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(Recognosce notum, ignotum inspice)

TRANSACTIONS

Edited by

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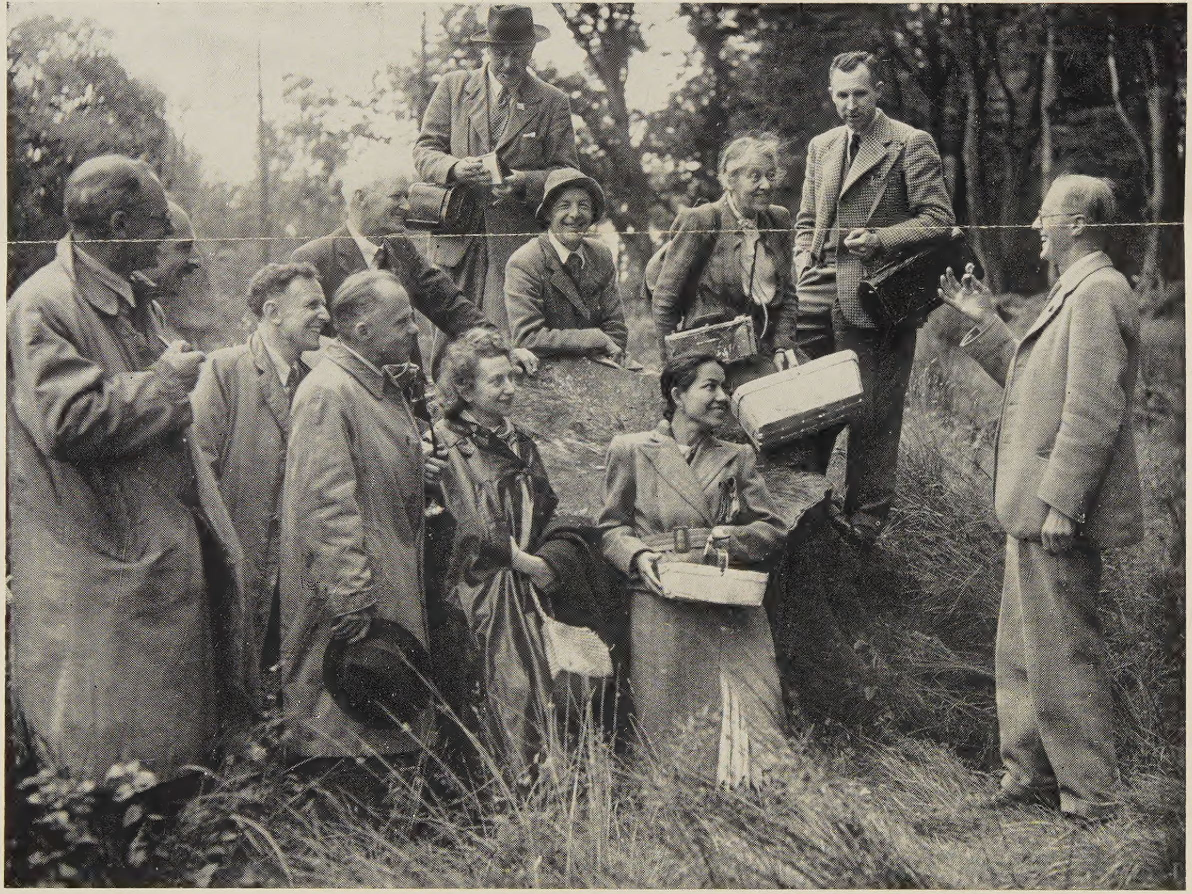

A. A. PEARSON (right) at the Belfast Foray, 1948.

With him (left to right) are: W. C. MOORE, E. C. LARGE, G. C. AINSWORTH, P. O'CONNOR, A. E. MUSKETT, E. N. CARROTHERS, MME M. LE GAL, K. ST G. CARTWRIGHT, MISS T. GOGOI, MISS E. M. BLACKWELL and J. COLHOUN.

(*Photograph by J. Roland Bainbridge, Belfast*)

ARTHUR ANSELM PEARSON

1874–1954

By the death of A. A. Pearson at Hindhead on 13 March 1954 our Society in particular, and mycology in general, suffered a very severe loss. He had been a member of the Society since 1911 and was Treasurer from 1919 to 1946, President in 1931 and again in 1952, the year of the joint meeting with the Société Mycologique de France. He was made an Honorary Member in 1946. He became a Fellow of the Linnaean Society in 1917, serving on their council from 1944 to 1948, and was President of the Yorkshire Naturalists Union in 1946. He was an active member of the Société Mycologique de France with whom he was almost as much at home as with the British Mycological Society; he was also a member of the Mycological Society of America. He was universally accepted as a leading authority on Agarics.

Arthur Anselm Pearson was born in London on 12 April 1874 and after having attended school in Belgium and spent a year at sea before the mast he joined the British Belting and Asbestos Ltd. of Cleckheaton, Yorks, when he was 15. He remained with this firm for the rest of his working life, becoming Director in 1912 and Chairman in 1937, an office he held until his death. He took much interest in the export trade of his firm and travelled extensively learning several languages so that he spoke and wrote fluently in French, German, Italian and Spanish.

In 1903 he married Miss Ellen Cusack and having started with golf as a hobby decided to take an interest in Natural History on the grounds that golf was taking him away from his family too much. At first he took up flowering plants but was soon attracted by the larger fungi and in 1910 received his first lessons in Agaricology from Dr J. Ramsbottom. He was keenly interested in music, especially song, particularly folk-song, and had a fine voice which he knew how to use. He was a member of the Oriana Madrigal Society and joined the English Folk Dance Society in 1924 and was particularly active during the building of Cecil Sharp House and helped with the publication of the Folksong Index. Until about two years ago he regularly attended a folk-dancing group and until very recently an accompanist used to visit his home once weekly for a singing session. He was especially fond of songs of Schubert and Brahms, and whilst in Germany in 1951 on a mycological visit sang Schubert songs to the assembly in such a fashion as to provoke the remark from one of them 'I never knew Englishmen could sing Schubert songs before!'

He was a devout Roman Catholic and was a loyal member of the Roman Catholic congregation wherever he lived. He sang in the choir of the church of St Charles Borromeo at Weybridge and later at the church of St Augustine at Tunbridge Wells. When he came to Hindhead he joined the congregation of the church of Our Lady of Lourdes at Haslemere. He was devoted to his family and is survived by his wife, three sons, of whom

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two are in business and one is a Professor of Classics in California, and a daughter.

He always interested himself in local activities, particularly after he retired. He was latterly a member of the Management Committee of Haslemere and District Hospital, a member of the Governing Body of Stoatley Rough School, Vice-President of Haslemere and District Youth Council and for some years Chairman of the Management Committee of Haslemere Educational Museum of which he became President in 1951 and where he gave lectures, led forays and to which he made many gifts. There he worked in conjunction with his old friend E. W. Swanton (Curator of the Museum) with whom he collected fungi during World War II to be tested at Oxford for antibiotics.

Although his published works are neither lengthy nor very numerous those describing species are notable for the excellence of the descriptions. His first papers published in the *Transactions* from 1918 onwards were on Resupinates in conjunction with Miss Wakefield. He very soon turned his attention to the Agarics and in 1919 described a new species of *Mycena*, *M. epipterygioides*. His first Presidential Address in 1931 was a summary of work done on Hymenomycetes in Europe, each country being treated separately. Then came some notes on Cooke's *Illustrations of British Fungi* and descriptions and comments on Agarics found in forays at Killarney (1936) and Aviemore (1938). 1938 saw the first of a series of five papers on 'New Records and Observations on Agarics', the most recent of which appeared in 1952.

In the Yorkshire *Naturalist* for 1945 a short paper was published on 'Diagnostic characters of Agarics', the first attempt in this country to enumerate modern methods of studying and determining Agarics. His Presidential Address to the Yorkshire Naturalists Union given in 1946 (published in 1947 in the same journal) was a critical survey of the Agarics in which some of the modern ideas of splitting Friesian genera were discussed and also some of the problems which arose out of the preparation of the 'Revised List of British Agarics and Boleti', later published in the *Transactions* in conjunction with Dr R. W. G. Dennis in 1948. Best known and perhaps most valuable of his publications are three short monographs in the form of a key with short tabulated descriptive notes on the genera *Russula* (1948, revised and reprinted in 1950), *Lactarius* (1950) and *Boletus* (1950), all in the Yorkshire *Naturalist*. Similar keys to *Cortinarius*, *Inocybe* and *Mycena* were prepared but not published by their author (although it is intended that they shall be published as soon as possible). In the case of *Cortinarius* the author would not publish the work because he felt it too incomplete, particularly in the sections *Telamonia* and *Hydrocybe* of which he said: 'No one has yet had the temerity really to work at these since Fries.' The *Inocybe* paper was at one time conceived to include one or two coloured plates showing as many of the species as possible on each plate, especially those which were closely related.

As a result of his travels abroad a list of Spanish fungi appeared in *Cavanillesia*, **4**, 1931. In 1948 he was invited to South Africa by the Federal Government to study Agarics, and as a result published a paper with coloured plates in the *Transactions* in 1950. In 1949 he went to Portugal—

again by invitation—but unfortunately struck a dry season. The results were however published in *Portugaliae Acta Biologica* (B), **3**, 1950, and included a description of *Marasmius cauvetii* Maire & Kühner, which was a new record for Europe but was found in Britain the same year.

It was in the field, however, that Pearson was such a dominant personality and charming companion. Whether at a Society's Foray, or on a private outing or starting at some unusually early hour abroad, he always stepped out with the same determined stride armed with a fearsome digging tool and a vasculum, ever ready to inspect and to comment on any agaric. In examining and naming specimens his patience was unlimited, the care and deliberation with which this was done exemplary, and his encouragement to the beginner most stimulating. Many have learnt much from the informal groups gathered round him in the woods. Even when besieged at home with parcels, sometimes relics in all states of freshness or decay, a courteous and helpful note was always quickly returned to the sender. His enthusiasm at the discovery of something of interest was always most encouraging to his correspondents

His approach to the description of Agarics was most methodical and the excellence of his descriptions owes much to the preparation of a printed form on which all details were entered under separate headings thus ensuring that no essential information was lacking. He was always prepared to adopt the changing ideas of the times on classification and methods of study but would accept no theory until it had been proved to his satisfaction—as he always said 'It is useless to speculate on such matters.' He was a pioneer in Britain in the adoption, or at least the introduction, of continental nomenclature and technique. He was quite prepared to modify the Friesian system, but was nevertheless very chary of abandoning it.

He gave valuable and willing service in many other ways at forays and other meetings. He was always ready to discuss mycological matters with the expert or the inexpert and rendered invaluable help in the management and organization of our Society. Those who were present at the joint meeting with the Société Mycologique de France in 1952 will long remember the skill with which he performed the arduous duties of Joint President and the ease and fluency with which he delivered his speeches in French—a fitting conclusion to a long term of service at forays at home and abroad. He had personal contacts with most of the leading agaricologists in other countries and his death, a loss to Mycology the world over, creates a gap which it will not be easy to fill. P. D. ORTON.

(*Note.* A portrait of A. A. Pearson forms the frontispiece to these *Transactions*, vol. **36**, 1953. His paintings, descriptions and collections of dried material have been deposited in the Herbarium, Royal Botanic Gardens, Kew. His library was bequeathed to the Linnaean Society of London, and the British Mycological Society.)

THE BASIDIOMYCETES OF SKOKHOLM ISLAND

I. ANNOTATED SPECIES LIST

By A. F. PARKER-RHODES

University Botanic Garden, University of Cambridge

(With 1 Text-figure)

Two hundred and fifty-six species of Eubasidiomycetes are listed as occurring on Skokholm Island, and notes are given of ecological data for species for which they are available. The list employs one new generic name, *Hydrabasidium*, and sixteen new combinations (see p. 338).

In any ecological investigation the proper foundation for subsequent work is a species list. In the author's investigation of the ecology of the higher fungi of Skokholm Island it was originally intended to begin by publishing such a list; but it soon became apparent that a list of species would take a long time to become full enough to be of any value. Owing partly to limitations in the observations themselves, but still more to the habits of the organisms concerned, there will always remain a not inconsiderable number of species unobserved. I do not refer to those which fructify at another time of year than the autumn, which are excluded altogether by the season at which the Island has been visited each year, but to those which might have been seen if they had only been fructifying in sufficient abundance in one year out of the seven which have passed since these observations began. Therefore this list has been held over till now; but it is already sufficiently long, and probably contains almost all the most abundant species and a majority of even the rarest. For the sake of brevity I have made no reference to the immediate evidence for the statements made about each species, which will be found in one or other of the author's papers in this series which have been appearing in the *New Phytologist*. I should perhaps explain that Skokholm is a small treeless island of Old Red Sandstone containing 240 acres and lying two miles off the coast of Pembrokeshire at the entrance to Milford Haven (see Fig. 1).

An apology is called for on account of a certain number of new combinations made in the nomenclature. This is a bad habit, but one which at the present time is unavoidable save by the increasingly irksome expedient of employing a demonstrably inadequate classification.

AURICULARIALES

AURICULARIACEI

1 **Platygloea effusa** Schröt. has been found occasionally on prostrate planks but it is unlikely to be maintaining itself without the importation of timber by man.

2 **Hirneola auricula** (Hooker) Karst. (*Auricularia auricula-judae* Bull. ex Fr.) grows on the elder tree in the Bird Observatory garden.

DACRYMYCETALES

Dacrymycetacei

3 **Dacrymyces stillatus** Nees ex Fr., ss. Martin (*D. deliquescens* Bull. ex Fr. ss. Rea) is fairly common on worked wood, fence posts, etc., and since it has been found on driftwood it has probably a self-propagating population on the island, though dependent on imported substrates.

4 **Ditiola radicata** (A. & S.) Fr. is also recorded from driftwood.

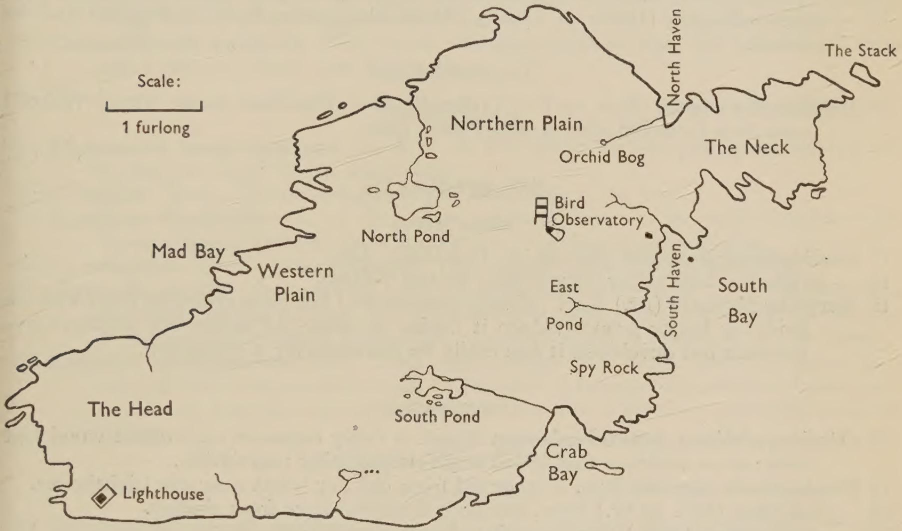

Fig. 1. Skokholm Island, showing the places referred to in the text, with scale.

TREMELLALES

Tremellacei

5 **Sebacina incrustans** (Pers. ex Fr.) Tul. was seen incrusting grass haulms in 1949, but has not been recorded since.

Tulasnellacei

6 **Hydrabasidium terrigenum** (Bres.) Parker-Rhodes* (*Ceratobasidium terrigenum* (Bres.) Rog.) is not uncommon on the humus under bracken though it is very easily overlooked on account of its grey colour.

THELEPHORALES

Hypochnacei

7 **Phlebiella vaga** (Fr.) Karst. (*Phlebia vaga* Fr.) is once recorded from a rotten plank.

8 **Soppitiella sphaerospora** (Maire) Parker-Rhodes (*Hypochnus sphaerosporus* Maire) appeared in 1953 on a dead stem of *Endymion*.

9 **Trechispora niveocremea** (Höhn. & Litsch.) Parker-Rhodes (*Corticium niveocremeum* Höhn. & Litsch.) has one record from rotten wood.

* The genus *Ceratobasidium* Rogers was regrettably founded on *C. calosporum* Rog. as type, and as this appears to be only a form of *Prototremella calospora* Boud. with aseptate basidia, the name *Ceratobasidium* cannot stand, though it is certainly a good genus. **Hydrabasidium** *gen.nov. est Ceratobasidium Rogersii circum typum Corticium atratum Bresadolae in statu Ceratobasidii calospori Rogersii reconstitutum.*

10 **Corticium byssinellum** Bourd. was seen several times on dead wood and on bracken petioles on the east side of the island in 1953. It is scarcely a true *Corticium*, on account of its ampullate hyphae and somewhat botryoid hymenium.

11 **Diplonema diademiferum** (Bourd. & Galz.) Parker-Rhodes (*Corticium diademiferum* Bourd. & Galz.) appeared on prostrate planks in 1952.

12 **Pellicularia vaga** (Berk. & Curt.) Rog. occurs occasionally on dead petioles of bracken.

13 — **pruinata** (Bres.) Rog. ex Lind. was also found on bracken in 1953.

14 **Botryohypochnus isabellinus** (Fr.) Donk has probably a consistent but fluctuating population on bracken debris, and also occurs on fence posts.

15 — **tulasnelloides** (Höhn. & Litsch.) Donk also occurs both on bracken and on wood.

THELEPHORACEI

16 **Prillieuxia caesia** (Pers. ex Fr.) Parker-Rhodes (*Hypochnus caesius* (Pers.) Wakef.) was once recorded from a fallen fence post.

POLYPORALES

MERULIACEI

17 **Coniophora puteana** (Schum. ex Fr.) Gray. And

18 — **arida** Fr. both occur occasionally on old timbers.

19 **Serpula pinastri** (Fr.) Rog. (*Merulius pinastri* Fr.) has been recorded from a plank bridging boggy ground; since it begins to sporulate before the poriform hymenium has developed it can easily be mistaken for a *Coniophora*.

CORTICIACEI

20 **Gloeocystidium praetermissum** Karst. is fairly common on worked wood but there is no evidence that it has a self-propagating population.

21 **Peniophora cremea** Bres. is recorded from old dry wood near the Lighthouse.

22 — **velutina** (DC. ex Fr.) Sacc. has been recorded once from timber.

23 — **molleriana** (Bres.) Sacc. was found at the same time. Also

24 — **byssoidea** (Pers. ex Fr.) Höhn. & Litsch. And

25 — **chordalis** Höhn. & Litsch.

26 — **pallidula** (Bres.) Sacc. occurred in the same locality (South Haven stream) in the following year. This close association of superficially similar species is paralleled several times in these records; in some cases at least the explanation is probably ecological. Many resupinate fungi are disseminated, it is believed, by insects, and the presence of cystidia may be connected with a particular mechanism of zoochory.

27 **Corticium epiphyllum** (Pers.) Donk (*C. arachnoideum* Berk.) may have a substantial population; it occurs on rabbit dung, but the mycelium often spreads also on to the humus. It also occurs on herbaceous debris.

28 — **byssinum** (Karst.) Mass. is represented by a large but very variable population which may fluctuate between 1000 and 100,000 (the largest estimated population for any species); on Skokholm it fructifies exclusively on dead bracken petioles, usually while they are still standing, and probably enters them as a weak parasite from the humus layers when they are dying back in the autumn.

29 — **farinellum** (Fr.) Höhn. & Litsch. (*Poria farinella* Fr.) was found twice in 1953 on worked wood.

30 — **terrestre** (DC. ex Fr.) Höhn. & Litsch. (*Poria terrestris* (DC.) Fr.) has been recorded once.

31 — **sambuci** (Pers.) Fr. probably has a self-maintaining population on woodwork in outbuildings, etc.; it is the commonest strictly lignicolous species on the island. It also occurs on driftwood and *Sambucus* twigs.

32 — **microsporum** (Karst.) Bourd. & Galz. occurs on worked wood, but it also occurs on dead bracken petioles, like *C. byssinum*, which like it has very small spores but is very much more abundant.

33 — **confluens** Fr. has been recorded once from fencing timber.

34 **Corticium tuberculatum** Karst. (*C. lacteum* Fr. ss. Rea) is more abundant, and occurs on driftwood, suggesting that it may be self-propagating on the island.

35 — **tessulatum** Cke. has been recorded from worked wood.

36 — **lividum** (Pers.) Fr. was recorded twice in 1952, one record being of a bisporous form with spores of approximately twice the normal volume.

37 — **ochraceofulvum** Bourd. & Galz. has been found on a spar of driftwood.

38 **Terana vellerea** (Ell. & Crag.) Parker-Rhodes was seen once on a fence post.

ODONTIACEI

39 **Grandinia mutabilis** (Pers. ex Fr.) Bourd. & Galz. has been recorded from planks lying on wet ground near North Pond.

40 **Ceratellopsis aculeata** (Pat.) Corn. (*Pistillaria aculeata* Pat.) has been found on a dead stem of *Juncus* near South Pond.

POLYPORACEI

41 **Podoporia sanguinolenta** (A. & S. ex Fr.) Donk yielded two specimens on old fencing in 1953; at the same time

42 — **cincta** (Berk.) Parker-Rhodes (*Poria cincta* Berk.) was seen.

43 **Coriolus versicolor** (L. ex Fr.) Quél. was seen once in the same locality overlooking South Haven; these species are scarcely self-propagating.

44 — **rancidus** (Bres.) Parker-Rhodes (*Poria rancida* Bres.) also grows there.

45 **Antrodia mollis** (Sommerf.) Karst. (*Trametes mollis* (Sommerf.) Fr.) has been seen on fallen fencing planks. The association of these five species together is attributable to the scarcity of suitable habitats for the Polyporacei on the island.

46 **Xylodon versiporum** (Pers.) Donk (*Irpex obliquus* Fr., *Poria vaporaria* ss. auct. Brit. etc.) is fairly frequent on wood, but in view of its great abundance elsewhere it cannot be inferred that it can maintain itself as a separate population on the island.

HYMENOCHAETALES

GANODERMATACEI

47 **Gloeophyllum saepiarium** (L. ex Fr.) Karst. was seen in 1952 on an old fencing plank.

AGARICALES

CLAVARIACEI

48 **Clavaria vermicularis** Fr. has a population of probably less than 100 in the grassland in the north of the island.

49 **Clavulinopsis corniculata** (Fr.) Corner is perhaps slightly more numerous, but its habit makes it easy to overlook.

50 — **helvola** (Fr.) Corner (*Clavaria inaequalis* Auct.) may have over 100 mycelia in various parts of the island; the variety **geoglossoides** Corner is also on record, but not from the wet ground which Corner indicates as its typical habitat.

51 — **fusiformis** (Fr.) Corner is represented by several hundred mycelia which are widely distributed, and there are three areas of significantly higher density; one in the north-east, one along the rocky ridges in the centre of the island, and along a former railway track in the south-west. It is estimated that it may attain to as much as 10% cover of the ground in the first area. The var. **ceranoides** Sm. is also found.

52 — **luteoalba** (Rea) Corner has only a few records, but so slender a fungus is easily overlooked; some specimens were of the var. **latispora** Corner.

53 — **pulchra** (Peck) Corner (*Clavaria persimilis* Cotton) was recorded once in 1953 near the Bird Observatory buildings.

CANTHARELLACEI

54 **Hygrophoropsis aurantiacus** (Wulf. ex Fr.) Maire (*Cantharellus aurantiacus* Fr.) has a fairly numerous population found both in grassland and under the more open brakes, which is rather unexpected in so typical a species of coniferous woodlands. It is also recorded from a worked fence post.

55 **Leptoglossum glaucum** (Batsch ex Fr.) Karst.* (*Leptotus glaucus* (Batsch ex Fr Maire) has been found on a dead bracken frond.

56 — **acerosum** (Fr.) Parker-Rhodes (*Pleurotus tremulus* Fr. sensu Rea) has an established but irregularly fruiting population in the brakes.

HYGROPHORACEI

57 **Hygrophorus camarophyllus** (A. & S. ex Fr.) Fr. (*Camarophyllus*) has been recorded from grassland near the Bird Observatory.

58 — **pratensis** (Pers.) Fr. (*Cam.*) has a population of around 100 mycelia, and may attain to about 5 % cover of the ground near the Bird Observatory. The variety **pallidus** B. & Br. also occurs, but none of the several other described varieties have yet been detected.

59 — **lacmus** Fr. (*Cam.*) has perhaps a few dozen mycelia, confined to the Northern Plain.

60 — **russocoriaceus** Berk. (*Cam.*) is recorded from near North Pond.

61 — **niveus** (Scop. ex Fr.) Fr. (*Cam.*) has about a hundred individuals, if one includes *H. virgineus* (Wulf. ex Fr.) Fr. which seems to me only a variety. The var. **fuscescens** Bres. also occurs; it is commonest in the north.

62 — **laetus** (Pers. ex Fr.) Fr. (*Hygrocybe*) has about the same population mainly in the eastern parts especially among the heather, which appears to be a frequent habitat of this species.

63 — **psittacinus** (Schaeff. ex Fr.) Fr. (*Hygroc.*) is fairly abundant, though its peak of fruiting probably falls rather later than most of the visits to the island so far made; it is found mainly in the east, and may have many hundreds of mycelia.

64 — **coccineus** (Schaeff. ex Fr.) Fr. (*Hygroc.*) is less numerous; the form which becomes yellow with age is also met with.

65 — **miniatus** Fr. (*Hygroc.*) has a regularly fruiting population of probably less than 100 mycelia, most of which occur in the wetter parts especially in the centre.

66 — **miniatus** var. **sphagnophilus** Peck (*turundus* var. *lepidus* Boud.) (*Hygroc.*) has been recorded from several patches of waterlogged ground; a specimen collected in 1947 had nearly spherical spores, but according to Pearson & Dennis (1948) the var. *sphaerosporus* Rea is really a *Laccaria*.

67 — **reai** Maire (*Hygroc.*) is rather more erratic; unlike the former species, most records are from the Northern Plain.

68 — **sciophanus** Fr. (*Hygroc.*) is not uncommon in eastern areas, especially in ditches and near watercourses.

69 — **ceraceus** (Wulf.) Fr. (*Hygroc.*) may have a dozen or so mycelia around the Bird Observatory, but has not yet been found elsewhere.

70 — **vitellinus** Fr. ss. Rea (*Hygroc.*) occurs in the region round South Haven but is not common.

71 — **citrinus** Rea (non ss. Lange) (*Hygroc.*) has been recorded once.

72 — **chlorophanus** (Fr.) Fr. (*Hygroc.*) is the most numerous species of this family on the island, with a population of the order of 1000 which falls into two main areas owing to its avoidance of the ill-drained soils in the centre. The density of occupation may attain to 5 % cover of the ground in the Northern Plain, but is less in the south-west. The forma **coccinella** with red marginal zone is not uncommon.

73 — **puniceus** Fr. (*Hygroc.*) was recorded in 1953.

74 — **conicus** (Scop. ex Fr.) Fr. (*Hygroc.*) may have about 100 mycelia mainly in the north; both two-spored and four-spored forms occur.

75 — **nigrescens** Quél. (non Lange) (*Hygroc.*) is recorded twice, from near North Pond and in Orchid Bog.

76 — **calyptraeformis** Berk. (*Hygroc.*) occurs in small numbers around South Haven. The var. **niveus** Cke. is also on record.

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* The genera *Leptotus* and *Leptoglossum* are of equal priority, but as the latter was first used by Ricken in 1920 for the combined genus, the former is no longer available in this sense.

77 **Hygrophorus unguinosus** Fr. (*Hygroc.*) appeared for the first time in 1953, when
78 — **foetens** Phill. (*Hygroc.*) was also observed.

TRICHOLOMATACEI

Tricholomatei

79 **Clitocybe fragrans** (Sow. ex Fr.) Kummer has about 1000 mycelia in grassland and in the brakes, being especially common in the *Rumex–Glechoma* association which occurs in parts of the latter; it attains to as much as 25 % cover according to estimation in one area. It has fructified abundantly only in 1950 so far.

80 — **diatreta** (Fr.) Quél. is abundant in the Armerietum, though like other species of this association it is erratic in fruiting; it may well have 100 or more mycelia and attain to quite a high cover, especially on the western cliffs; this preference is very unusual, and suggests a close connexion with the *Armeria* which also flourishes there. Specimens have been found intermediate between this species and the last, sometimes with no spores, which may well be hybrids.

81 — **candicans** (Fr.) Quél. is scarcely so numerous, and distributed in all parts of the island; a dwarf form on rabbit dung has also been seen.

82 — **ericetorum** (Bull. ex Fr.) Quél. fructified abundantly in 1948 but has not been observed since; its population of some hundreds is widely distributed, but is especially concentrated round South Haven (where it may attain 10 % cover) and Spy Rock.

83 — **rivulosa** (Pers. ex Fr.) Quél. has been seen once, among heather; but a few rings of the small form **neptunea** Fr. are rather regular in fruiting near the Bird Observatory.

84 — **flaccida** (Sow. ex Fr.) Quél. has been seen in the Neck.

85 — **fritilliformis** (Lasch) Quél. gave two records in 1953.

86 **Tricholoma cuneifolium** (Fr.) Quél. has been seen occasionally, especially in 1953, in short grass.

87 — **carneum** (Bull. ex Fr.) Quél. is abundant, with several hundred mycelia and attaining perhaps 15 % cover around the Bird Observatory; the majority of specimens, especially in the peak fruiting year, were of the hygrophanous variety called *T. persicolor* Fr., which however can hardly be maintained as a distinct species.

88 — **rutilans** (Schaeff. ex Fr.) Kummer var. **variegatum** (Scop. ex Fr.) Quél. (*Tricholomopsis*) is an unexpected inhabitant of the brakes, being on record from two well-separated localities and so probably having a small established population; neither case gave any indication of lignicolous habit.

Collybiei

89 **Collybia succinea** (Fr.) Kumm. may have as many as 100 mycelia, most of them being found among *Calluna*. It has only been seen in any numbers in 1950.

90 — **coracina** (Fr.) Quél. (*Lyophyllum*) has one record, from under bracken.

91 — **clusilis** (Fr.) Quél. nec Kühn. & Rom., is also recorded.

92 **Omphalina umbellifera** (L. ex Fr.) Quél.* has been found occasionally; one record is of the type, one of the pale var. **nivea** Fr., and two of the dark var. **pyriformis** (Pers.) Fr.

93 — **retosta** (Fr.) Quél. has been found in North Haven gully.

94 — **hepatica** (Batsch ex Fr.) Quél. was found under bracken near South Bay in 1950.

95 — **fibula** (Bull. ex Fr.) Quél. is very common among acrocarpous mosses, and its population may perhaps reach some hundreds of small mycelia, though the total area of its habitat is quite small. It is a rather constantly fruiting species, having been missed only in 1949; the variety **nivalis** Fr., much less closely bound to mosses, was frequent in 1953.

96 — **swartzii** (Fr.) Sing. has a much smaller population, occurring in very similar habitats to the last; both species like sheltered places, ditches and drains being especially favoured.

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* The generic name *Omphalia* (Fr.) Staude is pre-empted by *Omphalia* L.

Mycenei

97 **Mycena filopes** (Bull. ex Fr.) Kumm., nec Kühn., occurs in the humus of the brak and has a population of about 10,000 mycelia, which may cover about half t available ground in some areas.

98 — **debilis** (Fr.) Quél. is recorded once from the same habitat.

99 — **cinerella** Karst., nec Kühn. & Rom., is occasionally found in the brakes; t Skokholm specimens never have the alkaline smell sometimes found with th species.

100 — **metata** (Fr.) Quél., nec Kühn., is probably fairly numerous, but there are t few records so far to enable its population to be estimated, owing to the lo efficiency of sampling in the brakes.

101 — **arcangeliana** Bres. may be quite common; the Skokholm records are Kühner's var. **oortiana**. It is remarkable that so common and distinctive species as this should have been overlooked in Britain until recently.

102 — **sanguinolenta** (A. & S. ex Fr.) Quél. has been extremely numerous with we over 10,000 mycelia and almost complete cover of the humus in some of th brakes, but it appeared to be declining in numbers until 1953 when it was aga very numerous. There is good evidence that the number of species in th Pteridietum on Skokholm is progressively increasing.

103 — **galopus** (Pers. ex Fr.) Kumm. may have 1000 or more mycelia; it is most ofte seen in the more open parts of the brakes.

104 — **polygramma** (Bull. ex Fr.) Kumm. var. **pumila** Lange occurs in the brakes the western parts of the island, not always in association with mosses whic Lange describes as the typical habitat.

105 — **vitilis** (Fr.) Quél., nec Kühn., is fairly abundant in the brakes; like *M. filop* with which it is sometimes confused it is a woodland species which seems to fin the Pteridietum an adequate substitute.

106 — **alcalina** (Fr.) Kumm. has probably more than 1000 mycelia, and is estimate to exceed 20 % cover in many of the brakes, growing in the upper humus laye on bracken debris in place of its typical habitat on coniferous twigs. Th Skokholm records are of Lange's var. **chlorinella**.

107 — **ammoniaca** (Fr.) Quél. is rather less abundant; but it occurs also in grass places where fairly sheltered; on Skokholm it always has an ammonia sme whereas on more acid soils it is frequently inodorous.

108 — **aetites** (Fr.) Quél., nec Kühn. & Rom., grows on the edges of the brakes, mainl in the eastern areas.

109 — **avenacea** (Fr.) Schröt. has been twice recorded, one record being of the smal spored var. **olivaceomarginata** (Mass.) Rea.

110 — **gypsea** (Fr.) Quél. is recorded from bracken humus.

111 — **atromarginata** (Fr.) Quél. was found under bracken in 1953.

112 — **olida** Maire was found growing inside an old bracken petiole.

113 — **pura** (Pers. ex Fr.) Kumm. is abundant both in the brakes and amon grass, especially where there is heather, outside. Its various preferences mak it impossible to estimate the population, but it can hardly be less tha 200.

114 — **amicta** (Fr.) Quél. (*Insiticia*) is very common, with probably over 10,000 myceli and attaining over 50 % cover in some at least of the brakes where it grows; i 1950 a majority of the records were of the so-called var. **iris** Berk., which wa seen again once in 1953.

115 — **corticola** (Schum. ex Fr.) Quél. (*Ins.*) was once found on a dead stem o *Potentilla* near East Pond.

116 — **hiemalis** (Osb.) Quél. (*Ins.*) occurs in the brakes and on remains of *Juncus* an may be fairly frequent.

117 — **speirea** (Fr.) Kühn. var. **camptophylla** (Berk.) Kühn. (*Ins.*) has been foun growing inside a dead petiole of *Pteridium*.

118 — **stylobates** (Pers. ex Fr.) Kumm. (*Ins.*) is very abundant in some years, but lik all quisquilicolous species its numbers no doubt fluctuate considerably; esti-mates during these observations have varied from less than a thousand to some tens of thousands. The grey var. **grisella** Stürm has also been seen.

119 **Mycena clavularis** (Fr.) Quél. (*Ins.*) may average 1000 mycelia or more; it has been found on dead stems of *Glechoma* and *Juncus*. A variety also occurs with more numerous gills (at least ten), somewhat intermediate between this species and the last but placed here on account of possessing a collar, which may conceivably be a hybrid. This agrees with the *Agaricus dilatatus* of Fries.

120 — **tenerrima** Berk., which normally grows on the bark of trees, has appeared on Skokholm on a cork plank washed up from the sea, a plausible substitute.

Marasmiei

121 **Marasmius foetidus** (Sow. ex Fr.) Fr. (*Micromphale*) was recorded from grassland in 1953.

122 — **impudicus** Fr. (*Micr.*) is fairly frequent, being found in grass especially on the edges of the bracken.

123 — **brassicolens** Rom. (*Micr.*) was found among close-cropped *Rumex acetosella* and *Polytrichum* spp. on a rocky hillock in 1953.

124 — **ingrata** (Schum. ex Fr.) Quél. (*Micr.*) one specimen is recorded from near East Pond which appears to belong here, differing from *M. foetidus* in the non-striate pileus, larger size, smaller spores, and characteristic mouldy smell.

125 **Omphalia abhorrens** B. & Br. (*Micromphale abhorrens* (B. & Br.) Parker-Rhodes) has probably over 1000 mycelia, though it has so far only been seen in 1950; like many of these erratic species it is probably commoner than is generally realized. One record is from a very sparse stand of bracken in the extreme west of the island, where few pteridietal species occur.

126 **Marasmius candidus** (Bolt. ex Fr.) Fr. ss. Sing. was very abundant in 1947, with perhaps over 10,000 mycelia growing on decayed petioles of bracken; but it has not been seen since 1948 and may perhaps have been exterminated in the unfavourable season of 1949.

127 — **amadelphus** (Bull. ex Fr.) Fr. occurs on dead stems of *Juncus* and *Pteridium*, but is not common.

128 — **graminum** (Lib.) Fr. may in favourable seasons attain to 10,000 mycelia, growing on the remains of grasses and other herbaceous plants and only occasionally on bracken; it is the most numerous species on the island not directly associated with this plant.

129 — **vaillantii** Fr. was found in an open brake in the south-west.

130 — **oreades** (Bolt. ex Fr.) Fr. (*Scorteus*) has about 1000 mycelia; few of them can be traced as recognizable rings, the largest of these being, in 1952, 15 yards in diameter and already rock-bound on three sides. The distribution is very uneven, perhaps on account of vegetative spread; near Spy Rock its mycelium covers perhaps 10% of the ground, and in the Neck perhaps 5%.

131 **Collybia exsculpta** (Fr.) Quél. (*Scorteus*) is recorded from bracken humus.

132 — **collina** (Fr.) Quél. (*Scorteus*) has been found in grassland once.

133 **Crinipellis stipitarius** (Fr.) Pat. has 100 or so mycelia which have so far fruited abundantly only in 1950, though they were fairly frequent also in 1953; the characteristic habitat is among *Rumex acetosella* and *Plantago coronopus* where there is little grass, and these conditions are most frequently realized on old tussocks and rocky slopes heavily grazed by rabbits. There are two well-separated areas of high density, which perhaps behave as genodemes, one in the Northern Plain and one in the western end of the island.

Cyphelloid species

134 **Lachnella villosa** (Pers. ex Fr.) Sing. (*Cyphella villosa* Fr.) was recorded from dead bracken in 1947, but has not been seen since.

135 — **filicina** (Karst.) Parker-Rhodes (*Cyphella villosa* var. *stenospora* Rea) has been recorded once from the same substrate.

136 — **citrispora** (Pil.) Parker-Rhodes (*Cyphella citrispora* Pil.), kindly identified for me by R. W. G. Dennis, has been recorded twice from standing bracken petioles.

137 **Calyptella capula** (Holmsk. ex Fr.) Quél. (*Cyphella capula* Fr.) has been observed occasionally and may well have a substantial population in some years; it has only been seen on bracken debris.

138 **Calyptella griseopallida** (Weinm.) Parker-Rhodes (*Cyphella griseopallida* (Wei Fr.) was very abundant on herbaceous remains of all kinds in the denser brak in 1953. Its population was then estimated to be tens of thousands, seco only to *Corticium byssinum* at the time.

Pleurotoid species

139 **Pleurotus mitis** (Pers. ex Fr.) Quél. (*Panellus*) has been observed on de bracken.

140 — **herbarum** Peck (*Pleurotellus herbarum* (Peck) Sing.) is recorded from livi *Glechoma*; it differs from the more familiar *P. hypnophilus* mainly in the broad pip-shaped spores and in parasitizing flowering plants rather than mosses.

141 — **dictyorrhizus** (DC. ex Fr.) Quél. (*Pleurotellus*) is a fairly common quisquilico whose population may reach 1000 in some years; there is some evidence th it may be a recent immigrant into the island.

142 — **pubescens** (Sow. ex Fr.) Quél. (*Pleurotellus*) was found twice in 1953.

143 — **applicatus** (Batsch ex Fr.) Quél. (*Resupinatus*) has been found on a pla bridging boggy ground above South Haven.

144 **Crepidotus variabilis** (Pers.) Quél. is fairly common especially in the brakes the centre of the island, growing on bracken debris and occasionally on ba soil in rabbit burrows, etc.; in most Skokholm specimens there are a proportio of spores of the '*Octojuga*' type, i.e. with longitudinal ridges and furrows, whic appear to be merely abortive variants showing up the longitudinal structure the incompletely filled spore-wall.

RHODOPHYLLACEI

145 **Entoloma jubatum** (Fr.) Kumm.* (*Luctuarius*) is recorded once from among *Calluna* in the Neck.

146 — **porphyrophaeum** (Fr.) Kumm. (*Luct.*) is a very characteristic species of th Callunetum, but its population can hardly reach 100 mycelia on account of th restricted area of this community.

147 — **wynnei** (B. & Br.) Quél. (*Luct.*) is also recorded in the same community.

148 — **speculum** (Fr.) Kumm. (*Hyporrhodius* ss. str.) has been recorded from unde bracken near East Pond.

149 — **nidorosum** (Fr.) Kumm. (*Hypor.*) is fairly common in grassy places, but th Skokholm specimens are always odourless; it was wrongly referred to in a earlier paper as *Leptonia solstitialis* (Fr.) Quél.

150 — **costatum** (Fr.) Kumm. (*Hypor.*) has one doubtful record.

151 — **sericeum** (Bull. ex Fr.) Kumm. (*Hypor.*) is fairly common in some years, havin perhaps 100 mycelia in grassland communities. It is hard to separate in the fiel from

152 **Nolanea staurospora** Bres. (*Hypor.*) which is about equally abundant if the record can be trusted; but there are indications that its period of peak fruiting is rathe later than the times of observations. The var. **umbonata** Fr. also occurs.

153 — **mammosa** (L. ex Fr.) Kumm. (*Hypor.*) has a smaller population in the grass lands of the eastern part of the island.

154 — **papillata** Bres. (*Hypor.*) has been recorded once.

155 — **juncea** (Fr.) Quél. (*Hypor.*) is found occasionally in boggy places with easter exposure; the var. **cuspidata** Fr. is also recorded.

156 — **rufocarnea** Berk. (*Hypor.*) has been recorded from under bracken.

157 — **infula** (Fr.) Quél. (*Leptonia*) has a few dozen mycelia among *Calluna* in th eastern areas.

158 — **verecunda** (Fr.) Quél. (*Lept.*) occurs under bracken but mostly in the more ope stands; it may have a population of some hundreds but the available record are insufficient for an exact estimate.

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* Strictly speaking the name *Entoloma* (Fr.) Kummer, 1871 (ss. Pearson and Dennis must give place to *Hyporrhodius* (Fr.) Staude, 1857. (Lectotypus: *Agaricus rhodopolius* Fr.

159 — **icterina** (Fr.) Quél. (*Lept.*) may reach 1000 mycelia in the same habitat.

160 **Leptonia molliuscula** (Lasch) Quél., ss. Kühn. & Rom., is the most abundant member of the family, and occurs in most of the brakes except in the west; its population must be of many thousands of mycelia. In previous publications it has been wrongly referred as *L. sericella* (Fr.) Quél.

161 — **anatina** (Fr.) Kumm. is on record both from grassland and the brakes.

162 — **chalybea** (Pers. ex Fr.) Kumm. is occasionally found in grassland.

163 — **serrulata** (Pers. ex Fr.) Kumm. is a fairly frequent grassland species; so far only the var. **laevipes** Berk. has been observed.

164 — **sarcita** (Fr.) Quél. (*Luctuarius*) occurs in swampy ground, especially around East Pond, where there may be a dozen mycelia.

165 **Claudopus byssisedus** (Pers. ex Fr.) Gill. has been found on bracken debris.

CORTINARIACEI

Strophariei

166 **Panaeolus campanulatus** (L. ex Fr.) Quél., ss. Lange nec Kühn. & Rom., is or at least has been abundant, with a few hundred mycelia in a rather uneven distribution with notable concentrations in various parts of the east coast and around North Pond; but in recent years it has been seen less often.

167 — **papilionaceus** (Bull. ex Fr.) Quél., ss. Rea, nec Kühn. & Rom., which is the most abundant of the grassland species of the island with well over 1000 mycelia; its distribution is closely similar to that of the former species. In some of its most favoured areas its mycelium is estimated to exceed 50% cover, which is improbably high for a ring-forming species unless it is homothallic (which would make for rapid recolonization of the vacant interiors of rings).

168 — **retirugis** (Fr.) Quél. is recorded once from horse dung.

169 — **acuminatus** (Fr.) Quél. I have not seen, but it was recorded in June 1947 by Mr J. Caffyn.

170 — **fimicola** (Fr.) Quél. is represented by two varieties, var. **cinctulus** (Bolt.) Cke. and var. **ater** Lange, both from horse dung; the latter is better regarded as a distinct species.

171 **Anellaria semiovata** (Sow. ex Fr.) Pearson & Dennis (*Panaeolus*) is common on horse dung, with a population which no doubt fluctuates considerably but may reach several hundreds at times; the two forms **separatus** (L. ex Fr.) and **minor** Lange are both on record. The var. **phalaenarum** Fr. is sometimes found where there is no trace of dung, and has also been seen on the small island of Gateholm where there are never any horses, so that it is doubtful whether it is obligately coprophilous as the type is.

172 **Psilocybe foenisecii** (Pers. ex Fr.) Quél. (*Panaeolus*) has a small population in damp grassland around South Pond.

173 — **ericaea** (Pers.) Quél. has not been found associated with *Sphagnum* which is its commonest habitat, nor with heather, but occurs occasionally on the waterlogged soils in the centre of the island.

174 — **callosa** (Fr.) Quél., ss. Quél.: to this species I refer a series of specimens very similar to *P. semilanceata* but widely expanding and obtuse, or with a small hemispherical papilla only, and much smaller spores. In most of them the stem base turns blue very easily when bruised.

175 — **semilanceata** (Fr.) Kumm. with probably a few hundred mycelia is fairly common in the central area, having fruited most abundantly in 1953; the records, however, are somewhat uncertain as it is a species very easy to overlook on account of its slender form and unobtrusive colouring. The var. **caerulescens** Cke. also occurs.

176 — **montana** (Fr.) Kumm. is a late fruiting species, and may be common though so far only observed in 1953; its typical habitat is sandy heaths. This species is known to French authors as *P. atrorufa*, which however was originally made by Fries a variety only. The true *atrorufa* is a woodland species and is apparently rare in Britain.

177 — **physaloides** (Bull. ex Fr.) Quél. was also observed for the first time in 1953.

178 **Psilocybe coprophila** (Bull. ex Fr.) Quél. though never abundant has been fair regularly recorded from rabbit dung.

179 — **scocholmica** Parker-Rhodes is the most abundant coprophilous agaric on t island, with a population which in a favourable year may reach 10,000; occurs on both horse and rabbit dung, but is most frequent on the latter.

180 — **bullacea** (Bull. ex Fr.) Kumm. is probably commoner than the last on hor dung, where its population may sometimes exceed 1000. It has only been four once on rabbit dung.

181 **Stropharia semiglobata** (Batsch ex Fr.) Quél. (*Psilocybe*) is a strictly metacopr philous species disseminated sometimes by the horse but almost certainly main by sheep on Skokholm, since it is so frequently found on or near the cliff slop which are a favourite haunt of the latter animals but avoided by the horse. Th population is probably about 100. The forma **stercoraria** Fr. also occurs.

182 — **merdaria** (Fr.) Quél. (*Psilocybe*) is a less numerous metacoprophilous specie The var. **major** Fr. is common around North Pond, one record being of a abnormal caespitose form.

183 — **luteonitens** (Vahl ex Fr.) Quél., ss. Rick. (*Psilocybe*), has been once recorde from a clump of grass among bracken.

184 — **coronilla** (Bull. ex Fr.) Quél. has been recorded once from a typical ruder habitat beside the Bird Observatory buildings.

Cortinariei

185 **Cortinarius cinnamomeus** (L. ex Fr.) Fr. has a small population in wet grass places, among *Juncus* etc.; it is probable that my observations have been to early for this species.

186 — **incisus** (Pers.) Fr. is recorded once from under bracken.

Naucoriei

187 **Tubaria embola** (Fr.) Gill. has been recorded once.

188 — **inquilina** (Fr.) Gill. (*Phaeota*) is recorded once from dead bracken.

189 — **anthracophila** Karst. (*Phae.*) has been found amongst moss under bracken; is not by any means obligately associated with burnt ground.

190 **Naucoria pediades** (Fr.) Kumm. (*Phae.*) has been seen in 1947 and 1953.

191 — **pusiola** (Fr.) Heim (*Phae.*) was rather common in the Northern Plain in 195

192 — **camerina** (Fr.) Quél. is a fairly frequent quisquilicolous species on dead bracke on the east side, and its population may at times reach 1000; the Skokhol specimens are all unusually small.

193 — **sideroides** (Bull. ex Fr.) Quél. is common in some of the brakes; it is strictly humicolous species, with a population less than 1000.

194 **Galera nana** Petri (*Naucoria*) may at times reach to 10,000 mycelia, and abounc in different years from the former though it affects the same habitat; it was no recorded before 1951, but may well have been overlooked.

195 — **clavata** Vel. (*Nauc.*) is occasionally recorded from the brakes.

196 — **hypnorum** (Schrank) Kumm. (*Nauc.*) is not uncommon in patches of moss o rocks in certain localities; unlike *Omphalina fibula* it shows no preference fo those of acrocarpous habit. Owing to its restricted habitat it is unlikely that it population exceeds 100.

197 — **tibiicystis** Atk. (*Nauc.*) is the only obligately sphagnophilous species so fa recorded and was seen in 1953, in Orchid Bog.

COPRINACEI

Bolbitiei

198 **Galera tenera** (Schaeff. ex Fr.) Kumm. (*Conocybe*) has a small population disperse in the central parts of the island. The vars. **subovalis** Kühn. and **convexa** Kühn. are also recorded, the former being fairly common.

199 — **antipus** (Lasch) Quél. (*Conoc.*) has been seen in the Neck.

200 — **spicula** (Lasch) Quél. (*Conoc.*) occurs occasionally in grass.

201 **Conocybe ochracea** (Kühn.) Kühn. & Rom. has been observed in several localities; both of Kühner's forms, the large-spored and the small-spored, are on record.

202 — **laricina** Kühn. has been found under bracken in the north-east.

203 — **coprophila** Kühn. has been observed on horse dung.

204 **Pholiota sphaleromorpha** (Bull. ex Fr.) Quél. (*Agrocybe*) has once been recorded from grassland.

Coprinei

205 **Hypholoma candolleana** (Fr.) Quél. (*Psathyrella*) grows on a plank used as a bridge near South Haven, but was not seen before 1952.

206 **Psathyrella badiophylla** (Rom.) Parker-Rhodes was found in a rabbit burrow in 1949.

207 — **fibrillosa** (Pers.) Pearson & Dennis has a small number of mycelia in heath communities, but has never been found fruiting plentifully.

208 — **stercoraria** (Kühn. & Joss.) Parker-Rhodes was found on horse dung in 1949; it was recorded than as *P. prona*, which is closely allied.

209 — **prona** (Fr.) Quél., ss. Kühn. & Rom., was found in a damp ditch in 1950.

210 **Coprinus ephemerus** (Bull. ex Fr.) Fr. has been found on horse and rabbit dung.

211 — **plicatilis** (Curt. ex Fr.) Fr. is probably common, but its fruiting season is rather late and it has accordingly been recorded only twice.

212 — **radians** (Desm.) Fr. or a related species is represented by its *Ozonium* mycelium, growing on thrown-out timber near the Lighthouse.

213 — **ephemeroides** (Bull. ex Fr.) Fr., var. **hendersonii** Berk. has been recorded several times from horse dung.

214 — **patouillardii** Quél. was recorded twice from the same substrate in 1953.

215 — **velox** Godey is probably the second most abundant species on rabbit dung on Skokholm, with a population which must exceed 1000 in favourable years, though estimates are likely to be too low with such a minute fungus; it is also recorded from sheep dung.

216 — **papillatus** (Batsch ex Fr.) Fr., var. **oxygenus** Fr. has twice been found on horse dung.

217 — **niveus** (Pers.) Fr. is one of the more constant coprophilous species, and has been observed in most seasons.

218 — **urticaecola** (B. & Br.) Buller occurs occasionally on dead stems of *Juncus* and similar plants; like other species of the Juncetum its frequency may well be underestimated, since it is difficult to sample this community adequately.

219 — **lagopus** Fr. was common on dung in 1947, but has only been seen once since then.

220 — **radiatus** (Bolt. ex Fr.) Fr. is probably always common on horse dung, but is very liable to be overlooked on account of its minute size and almost transparent pileus.

221 — **atramentarius** (Bull. ex Fr.) Fr. was seen at the Bird Observatory in 1947.

AGARICACEI

Agaricei

222 **Agaricus arvensis** Schaeff. ex Fr.* is represented by some hundreds of rather erratically fructifying mycelia found mainly round the south and east coasts, but with a particular concentration on the rocky ridge behind the Bird Observatory, where it may attain to 5 % cover, and in the Neck.

223 — **stramineus** (Møll.) Møll. may perhaps have 100 mycelia widely dispersed about the island; but it has so far only been observed in 1950.

224 — **sylvicola** Vitt. occurs in small numbers, on the Neck, and in the Lavateretum on the Stack rock, being the only species so far recorded from the latter locality.

225 — **xanthodermus** Genev. is represented by two records of a rather aberrant form with a coarsely scaly pileus and foetid-smelling mycelium, both from cliff-top situations.

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* The generic name *Psalliota* (Fr.) Kumm. used by Pearson & Dennis (1948) is unavailable since *Agaricus* L. ex Fr. has priority with the same lectotype.

226 **Agaricus meleagroides** Møll. is represented by one record from grass at the edge of the Pteridietum near South Haven.

227 — **comtulus** Fr. is common, especially in the eastern parts and in the Northern Plain, where it may have a hundred or so mycelia with their greatest concentration near North Haven. There are apparently two forms, one rapidly turning yellow when bruised, the other only slowly changing.

228 — **semotus** Fr. is one of the commonest species of the Armerietum, though probably short of 100 mycelia. Several varieties occur, which Møller (1952) makes distinct species; these are however very closely related and appear to have identical ecological requirements. The *Psalliota amethystina* of Quélet, which Møller thinks is the true *semotus*, is perhaps the commonest, but var. **xantholepis** (Møll.) is also frequent, and var. **purpurascens** (Møll.) has been recorded once.

229 — **campestris** L. ex Fr., ss. Møll. is less common; most records are of the var. **praticola** (Vitt.) Fr., whose favourite habitat is cliff-top situations where there is no *Armeria*, and **A. porphyrius** (Møll.) Møll. which I regard as only a variety, is also on record.

230 — **silvaticus** (Schaeff.) Fr. is fairly common; the vars. **haemorrhoidarius** (Schulz.) Møll. and **pallidus** Møll. have also been observed once each. Many records of this species however are of old specimens, which may easily be confused with:—

231 — **langei** (Møll.) Møll. (*Psalliota haemorrhoidaria* ss. Lange) which occurs rather regularly in the neighbourhood of Mad Bay; in view of the distribution however it is not certain whether all the records may not be from one very large ring.

232 — **variegans** Møll. is represented by perhaps 100 mycelia, especially around South Bay; but the status of this species is open to doubt, since it appears to intergrade with *A. langei* and perhaps also with *A. impudicus*.

233 — **impudicus** (Rea) Parker-Rhodes is perhaps about equally numerous, and has a similar distribution, though the fruiting of the two species is not correlated closely; if they are really distinct, they may be presumed to be in active competition.

234 — **hortensis** Cke. appeared in some numbers in 1948 and again in 1953; several different forms have been recorded, including the type, the form **albidus** Lange, f. **fulvaster** Viv., and a grey squamulose form. In view of the presumably apomictic status of these forms it is perhaps scarcely worth naming them. The total population may be about 100.

235 — **spissicaulis** Møll.: a fungus which may be a dwarf form of this species has been rather constantly recorded, especially along the former railway track.

236 **Lepiota procera** (Scop. ex Fr.) Gray is the largest and most conspicuous fungus on the island; it probably has less than 100 mycelia in all, though quite a large proportion of these may have been already recorded. Many of these take the form of well-defined rings with diameters varying from 20 to 60 ft. The highest density is observed in the Callunetum, but it also occurs in grassland and open brakes.

237 — **excoriata** (Schaeff. ex Fr.) Quél. has fewer mycelia, all in the Callunetum.

238 — **naucina** (Fr.) Quél. (*Chamaeota*) is a species of the Armerietum, being especially common in the extreme west of the island, where few fungi except those of this habit can flourish. In addition to the type, forms answering to the species **L. holosericea** (Fr.) Quél. and **L. scobinella** (Fr.) Quél. are also found, but they intergrade so closely that they can hardly be distinct species here. This fungus agrees in all essential characters with the genus *Agaricus* except in spore colour.

239 — **clypeolaria** (Bull. ex Fr.) Kumm. (*Lepiotula*) is fairly common in grassland in the neighbourhood of South Haven.

240 — **laevigata** Lange (*Lep.*) occurs in the heath near East Pond.

241 — **felina** (Pers.) Quél. (*Lep.*) is very abundant in the brakes, with perhaps 10,000 mycelia, and covering over a third of the ground in some areas. It appears to be strictly humicolous, but the precise level in which the mycelium grows has not been ascertained.

42 **Lepiota georginae** Sm. (*Lep.*) is estimated to be the most abundant of the strictly perennial species on the island at the present time, and must attain to nearly 100% cover of its humus layer in many areas of Pteridietum; it is however a very irregular fruiter, having been sufficiently abundant for statistical assessment only in 1950, though it has also been recorded in two other seasons. It is probably this irregularity which accounts for its being regarded as a rare species, and there is no reason to think that its status on Skokholm is exceptional.

Pluteei

43 **Volvaria speciosa** (Fr.) Kumm. has been recorded from an old nettle bed at the Bird Observatory; it is a typical ruderal species. It is unlikely that it has many mycelia on the island, where such habitats are few.

LYCOPERDALES

NIDULARIACEI

Nidulariei

244 **Nidularia broomei** (Mass.) Zeller was recorded several times in 1953 from bracken debris.

Sphaerobolei

245 **Sphaerobolus stellatus** Tode ex Pers. is a common quisquilicolous species of dead bracken and occasionally other debris; the evidence suggests that it is rapidly increasing in numbers, the records for seven successive years being 0, 1, 0, 2, 2, 20, 41: the last two figures include about 50% of sterile mycelia, which in this species can be fairly certainly recognized. The present population must be well over 10,000.

246 — **stercorarius** Fr., ss. Parker-Rhodes, has occasionally been seen on rabbit dung.

247 — **tubulosus** Fr., ss. Lorenz., has been recorded from sheep dung.

LYCOPERDACEI

Lycoperdei

248 **Calvatia bovista** (Pers.) Th. Fr. (= *C. caelata* ss. Rea) has a small population in grassland.

249 — **excipuliformis** (Pers.) Perdeck, non Rea (= *C. saccata* (Vahl) Fr.) has been recorded three times, it is a grassland species which occurs also in *Armeria*.

250 — **depressa** (Bon.) Lloyd has a large population of several hundreds widely scattered, but with two distinct areas of higher density, one around North Haven (where it may reach 10% cover) and one near Crab Bay.

251 — **candida** (Pers.) Lloyd is probably slightly less common, though in some years it has fruited more abundantly; it is very evenly distributed over the island, with an average cover of about 2%. It is difficult to resist the conclusion that these two species are in active competition.

252 **Lycoperdon umbrinum** Pers. ss. Rea is rather common in the Callunetum and occurs also in the Armerietum, but scarcely outside these two communities. Its population is probably below 100, but it is recorded most seasons.

253 — **ericetorum** Pers. sensu Perdeck. (= *L. polymorphum* Vitt.) is more numerous, and is especially common in places with an eastern exposure; a majority of records are of the form **cepaeforme** which has no externally visible sterile base.

254 — **pusillum** Batsch ex Pers. ss. Rea has been frequently recorded in recent years, always in the Armerietum where it is widely distributed; it often appears to be attached to the decaying stems. Most specimens have a distinctive rufous colour, reminiscent of that of *L. pyriforme* var. *tessulatum* Pers.

Bovistei

255 **Bovista plumbea** Pers. ex Pers. has a population of some hundreds in all associations very widely distributed.

256 — **nigrescens** Pers. is much less common, but also is recorded from all parts of the island; these two species also appear to be in direct competition, but they probably have different ecological requirements, since their fruiting habits are dissimilar.

Note on estimates of population and cover

The meaning attached to the term 'mycelium' in perennial humicolous species is determined by the fact that in the absence of clear evidence to the contrary two fruit bodies in grassland were deemed to spring from different mycelia if they were 5 yards or more apart; while in the brakes the minimal separation has been taken as 1 yard. This does not affect the estimate of percentage cover, which however depends on the assumption that, again in the absence of contrary evidence, the radial width of the mycelium in a ring is 1 yard, including the inner zone not accessible for growth of the same species, and that the minimum radius of a fruiting mycelium is 1 yard, so that a ring not exceeding 4 yards in diameter 'covers' effectively the whole of its area. The humicolous species of the brakes are assumed not to form rings unless there is positive evidence that they do. Failure of these assumptions would usually lead to percentage cover being over-estimated.

List of new combinations

After each combination is given the cause of the transfer; all binomials thus mentioned are types or lectotypes of their respective genera.

233 *AGARICUS IMPUDICUS* (Rea) comb.nov., basonym *Psalliota impudica* Rea, *Trans. Brit. mycol. Soc.* **15**, 37, 1932. Replacing invalid generic name.

138 *CALYPTELLA GRISEOPALLIDA* (Weinm.) comb.nov., basonym *Cyphella griseopallida* Weinmann, *Hymenomycetes et Gasteromycetes hucusque in Imperio Rossico observatos*, 1836. Replacing invalid generic name; resembles *Calyptella capula* (Holmsk. ex Fr.) Quél. and differs from *Lachnella alboviolascens* (A. & S.) Fr., etc., in the morphology of the cuticular hairs.

44 *CORIOLUS RANCIDUS* (Bres.) comb.nov., basonym *Poria rancida* Bresadola, *Fungh. Trid.*, Pl. 208, fig. 1 (1898). Differs from *Poria medulla-panis* (Jacq. ex Fr.) Cke. and resembles *Coriolus zonatus* (Fr.) Quél. in possessing generative, skeletal, and binding hyphae, and in having no intercellular potash-soluble colloidal matter.

11 *DIPLONEMA DIADEMIFERUM* (Bourd. & Galz.) comb.nov., basonym *Corticium diademiferum* Bourdot & Galzin, *Bull. Soc. mycol. Fr.* **27**, 244, 1911. Differs from *Corticium confluens* Fr. and resembles *Diplonema sordidum* Karst. in the want of a well-defined subhymenium and the presence of non-basidiophorous hyphae among the basidia.

6 *HYDRABASIDIUM TERRIGENUM* (Bres.) comb.nov., basonym *Corticium terrigenum* Bresadola, *Ann. mycol., Berl.*, **1**, 98, 1903. Replacing invalid generic name *Ceratobasidium* Rogers.

136 *LACHNELLA CITRISPORA* (Pilát) comb.nov., basonym *Cyphella citrispora* Pilát, *Ann. mycol., Berl.*, **22**, 209, 1924. Replacing invalid generic name; resembles *Lachnella alboviolacea* (A. & S.) Fr. in the morphology of the cuticular hairs.

135 *LACHNELLA FILICINA* (Karst.) comb. nov., basonym *Cyphella filicina* Karsten, *Not. Soc. Fenn.* **11**, 220. For the same cause.

56 *LEPTOGLOSSUM ACEROSUM* (Fr.) comb.nov., basonym *Agaricus acerosus* Fries, *Icones selectae Hymenomycetum nondum delineatorum*, Pl. 89, fig. 2, 1870. Resembles *Leptoglossum muscigenum* (Bull. ex Fr.) Karst. in the thick gelatinous hyphal walls, the morphology of the trama, and the pigmentation.

125 *MICROMPHALE ABHORRENS* (B. & Br.) comb.nov., basonym *Omphalia abhorrens* Berkeley & Broome, sensu Rea, *British Basidiomycetae*, p. 430, 1922. Resembles *Micromphale foetida* (Pers. ex Fr.) Sing. in reviving after drying, the simple hyphoid cuticle, the short down-tapered stem, and odour of mercaptan.

42 *PODOPORIA CINCTA* (Berk.) comb.nov., basonym *Poria cincta* Berkeley, *Grevillea*, 1872. Resembles *Podoporia confluens* Karst. in its maculate habit, restricted lateral growth, and presence of intercellular potash-soluble colloidal matter.

16 *PRILLIEUXIA CAESIA* (Pers. ex Fr.) comb.nov., basonym *Thelephora caesia* Persoon expositu Fries, *Systema Mycologicum*, 1821. Differs from *Tomentella ferruginea* (Fr.) Pat. and resembles *Prillieuxia faginea* (Britz.) Sacc. in the presence of a well-defined subhymenium of narrower hyphae, and a regular hymenial palisade.

206 *PSATHYRELLA BADIOPHYLLA* (Rom.) comb.nov., basonym *Drosophila badiophylla* Romagnesi, in Kühner & Romagnesi, *Flore Analytique des Champignons supérieurs*, 1953. Replacing invalid generic name.

208 *PSATHYRELLA STERCORARIA* (Rom.) comb.nov., basonym *Drosophila stercoraria* Romagnesi, in Kühner & Romagnesi, *Flore Analytique des Champignons supérieurs*, 1953. Replacing invalid generic name.

8 *SOPPITTIELLA SPHAEROSPORA* (Maire) comb.nov., basonym *Hypochnus sphaerosporus* Maire, *Bull. Soc. mycol. Fr.* **21**, 164, 1905. Differs from *Tomentella ferruginea* (Fr.) Pat. in the hyaline spores and small irregular dilated hyaline hyphae, and from *Phlebiella vaga* (Fr.) Karst. in the former character, and resembles *Soppittiella fastidiosa* (Pers. ex Fr.) Mass. in both.

38 *TERANA VELLEREA* (Ell. & Crag.) comb.nov., basonym *Corticium vellereum* Ellis & Cragin, *Bull. Washb. Coll. Lab.* (*Nat. Hist.*), **1**, 1885. Differs from *Corticium confluens* Fr. and resembles *Terana caerulea* (Schrad. ex Fr.) O. Kuntze in the receptacle being composed of relatively thick-walled, smooth, little-branched, non-agglutinated hyphae.

9 *TRECHISPORA NIVEOCREMEA* (H. & L.) comb.nov., basonym *Corticium niveocremeum* von Höhnel & Litschauer, *Sitzb. Akad. Wiss. Wien* (*Math./Nat.* Ser. II*a*), **117**, 1106, 1908. Resembles *Trechispora onusta* Karst. in the urniform basidia, it being supposed that the final form of the basidia is of more consequence in this respect than the detail of their development.

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PECTINASE PRODUCTION BY *BOTRYTIS CINEREA* AND *PYTHIUM DEBARYANUM*

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(With 2 Text-figures)

For *Botrytis cinerea* increase of asparagin concentration leads to an increase in growth and enzyme production. Increase of sucrose or glucose concentration results in a greatly increased growth but in a decrease of enzyme production. With a high C/N ratio an inverse correlation between mycelium growth and enzymic activity is shown; if the C/N ratio is reduced by using higher asparagin concentrations this correlation is direct. Increase of $MgSO_4$ concentration results in a marked decrease in enzyme production. The retarding effect is also shown if $MgSO_4$ solutions are used to dilute enzyme preparations.

For *Pythium debaryanum* pectinase secretion is highest on dilute potato decoctions and on a synthetic medium N (see p. 348). The nitrogenous constituents of the synthetic medium appear of greater importance for enzyme production than the carbohydrate constituents. The C/N ratio effects pectinase activity. Pectinase activity increases with decreasing concentration of $MgSO_4$ and K_3PO_4 and decreases when the concentration of these salts is increased. The optimum pH for pectinase activity in Medium N is on the alkaline side of neutrality.

For *Botrytis cinerea* pectin favours pectinase secretion while for *Pythium debaryanum* pectin not only does not increase pectinase production but its presence in a medium otherwise suitable for enzyme production is harmful.

Introduction

Various workers have examined the ability of certain micro-organisms to produce pectinase enzyme in order to correlate parasitic capacity with enzyme secretion.

Brown (1915, 1917) germinated spores of *Botrytis cinerea* on glass plates, and obtained preparations of the enzyme either by grinding and extracting the germinated spores (endoenzyme) or by use of the liquid in which germination had taken place (exoenzyme). A large variety of media—potato extract, turnip extract, grape juice—proved to be quite satisfactory. In similar studies on *Phytophthora erythroseptica* and *Pythium debaryanum*, Chona (1932) used pieces of fresh potato and after attack had proceeded for some time, squeezed out the sap from the rotted tissue. In this way quite active enzymic preparations were obtained. Menon (1934) also worked on *P. debaryanum* and obtained active enzymic preparations by growth in autoclaved potato tissue. Fernando (1937), in a study of pectinase from *Pythium*, used Menon's method and found it to be quite satisfactory. This paper reports further studies on the pectinase production by *Botrytis cinerea* and *Pythium debaryanum*.

Botrytis cinerea enzyme was prepared by using Brown's method (1915). 0·1 ml. of centrifuged spores was suspended in 10 ml. of the medium and sown on levelled glass plates. The spores were left to germinate for 2 days at laboratory temperature. The liquid medium was used as such to determine the exoenzyme activity. For this purpose, thin potato disks were placed in the medium and the time required for complete loss of coherence of the disks was noted; the activity was inversely proportional to the maceration time.

Endoenzyme preparations were made by drying the mycelium and grinding it with an equal weight of clean dry sand. For testing the endoenzyme activity, 0·2 g. of this powder was extracted in 3 ml. of water.

In order to correlate the results obtained at different times, a stock material was prepared from which an enzyme solution of standard strength could be prepared at will. This standard enzyme was the endoenzyme prepared after fungal growth in the following Medium A: glucose, 25 g.; asparagin, 10 g.; KH_2PO_4, 5 g.; $MgSO_4.7H_2O$, 2·5 g.; water, 1000 ml. In each experiment a sample extract of the standard endoenzyme was included, and the activity of this was taken as 100.

Pythium debaryanum enzyme was prepared by growing an isolate from potato for 10 days in small flasks of potato juice or extract and then determining enzymic activity of the culture fluid as already described.

I. THE EFFECT OF CULTURAL CONDITIONS ON THE PRODUCTION OF PECTINASE BY *BOTRYTIS CINEREA*

Effect of asparagin concentration on pectinase activity

Table 1 shows the effects of a range of asparagin concentrations, varying from 0·0 (water) to 5·0 % on growth, pH and enzyme activity.

Table 1. *Effect of asparagin on pectinase enzyme production by* Botrytis cinerea

Asparagin %	Initial pH	Final pH	Av. germ tube length (mm.)	% germination	Exoenzyme activity
0·0 (water)	7·0	7·1	—	Slight	6
0·019	6·8	6·85	—	Slight	6
0·078	5·9	7·0	0·033	22	15
0·312	5·2	7·3	0·06	26	25
1·25	5·2	7·2	0·06	46	38
5·0	5·2	6·9	0·1	70	33

It is clear that increased concentration of asparagin in the medium produces an acid reaction and that as growth proceeds the reaction tends towards neutrality. Increased asparagin concentration leads to greater growth and greater activity of the exoenzyme.

Similar results were obtained from another experiment in which a basal salt solution of KH_2PO_4 and $MgSO_4$ was used.

Effect of carbohydrate concentration on pectinase activity

Two carbohydrates, sucrose and glucose were tested. It is seen from Table 2 that, though there is a substantial amount of growth with sucrose, secretion of exoenzyme is at a low level throughout; and there is a suggestion that it falls somewhat as the concentration of sugar is increased.

Similar results were obtained when increasing glucose concentrations were used, and an experiment in which varying amounts of glucose were added to Medium A showed that for each unit of mycelium produced (on a dry-weight basis) less exoenzyme is formed at higher glucose concentrations, e.g. for glucose concentrations of 0·1 and 2·0 % the mycelial dry weights and enzyme activities were 65 mg. and 27 and 324 mg. and 45, respectively.

Table 2. *Effect of sucrose on pectinase enzyme production by* Botrytis cinerea

% sucrose	Wet vol. (ml.) mycelium (per plate)	Activity
0·0 (water)	0·1	3·0
0·019	0·1	3·0
0·078	0·3	2·9
0·312	0·4	2·7
1·25	0·22	2·7
5	0·2	2·5

Effect of glucose and asparagin concentration on pectinase activity

Table 3 gives the amounts of growth and the corresponding exoenzyme activities on a basal medium supplemented with varied amounts of glucose and asparagin.

Table 3. *Effect of glucose and asparagin on pectinase production by* Botrytis cinerea

Asparagin...	0·005 %		0·25 %		1 %	
% glucose	Wet vol. mycelium (ml.)	Activity	Wet vol. mycelium (ml.)	Activity	Wet vol. mycelium (ml.)	Activity
0·01	0·01	6·3	0·01	7	0·1	8
0·04	0·1	5·5	0·1	20	0·25	36
0·2	0·6	4·7	0·4	33	0·3	36
1·0	1·3	2·3	1·2	60	1·0	100
5·0	1·5	2·4	2·0	33	2·5	100

Basal medium KH_2PO_4 0·05 %, $MgSO_4.7H_2O$ 0·025 %.

Increase in glucose concentration increases growth, and the amount of growth is substantially as great (other things being equal) at the lower as at the higher levels of asparagin. With minimal asparagin concentration, exoenzymic activity is very slight and tends to fall with increasing glucose concentration; with higher asparagin concentration, enzymic activity increases with increasing glucose concentration.

Table 3 also shows that increase of glucose concentration from 1 to 5 % increases growth but does not lead to a corresponding increase in enzyme activity which is even reduced when 0·25 % asparagin is used.

This effect was analysed more fully in series of experiments with two media of the following composition:

	Medium 1 (%)	Medium 2 (%)
Glucose	1·0	5·0
Asparagin	0·25	0·25
KH_2PO_4	0·05	0·05
$MgSO_4.7H_2O$	0·025	0·025

It was shown that the activity of exoenzyme on Medium 1 was much higher than that on Medium 2. The experiment was repeated several times, and the difference between exoenzymic activity of the two media was found to be significant. Subsidiary experiments showed that the low activity on Medium 2 was due to less enzyme being secreted in this medium and not to enzyme-retarding substances. Endoenzyme activity was determined after the fungal growth on both media. It was shown that the endoenzyme on Medium 2 was greater—in contrast to exoenzyme activity—than that on Medium 1, but the difference was not significant.

Effect of increased concentrations of magnesium sulphate added to a basal medium of 1·25 % asparagin is shown in Table 4. It is clear that increase of $MgSO_4$ concentration sharply reduces exoenzymic activity.

Table 4. *Effect of* $MgSO_4$ *on pectinase production by* Botrytis cinerea

Asparagin 1·25 %	Enzyme activity
0·0	100
0·05 % $MgSO_4.7H_2O$	42
0·25 % $MgSO_4.7H_2O$	12
1·25 % $MgSO_4.7H_2O$	3

The effect of magnesium sulphate on enzyme activity, as apart from enzymic production, was examined by comparing the effects on enzyme activity of water and two concentrations of magnesium sulphate when these were used to dilute enzyme preparations. Two such preparations were used, made from cultures on A (standard medium) and A/10 media (Table 5).

Table 5. *Effect of* $MgSO_4$ *on pectinase activity*

		0		1/4		1/16		1/64	
Exoenzyme preparations	Diluents	% $MgSO_4$	Activity	% $MgSO_4$	Activity	% $MgSO_4$	Activity	% $MgSO_4$	Activity
From medium A	Water	0·25	200	0·06	88	0·015	44	0·004	30
	0·025 % $MgSO_4$	0·25	200	0·25	44	0·25	33	0·25	15
	0·5 % $MgSO_4$	0·25	200	0·44	33	0·48	22	0·5	7
From medium A/10	Water	0·025	100	0·006	60	0·002	24	0·0004	13
	0·5 % $MgSO_4$	0·025	100	0·38	12	0·48	3	0·49	3

It is clear that the retarding effect of $MgSO_4$ is greater when dilution of enzyme goes parallel with increase of concentration of magnesium sulphate.

Effect of pH *value on pectinase activity*

A range of pH values was established by the addition of N/10-NaOH and N/10-HCl to Medium A. The activity of each enzyme solution was estimated and compared with that of the enzyme solution to which no acid or alkali was added. The results are represented in Fig. 1, from which it is clear that the optimum for enzyme activity is on the acid side of neutrality and that activity decreases both with increasing and decreasing pH values.

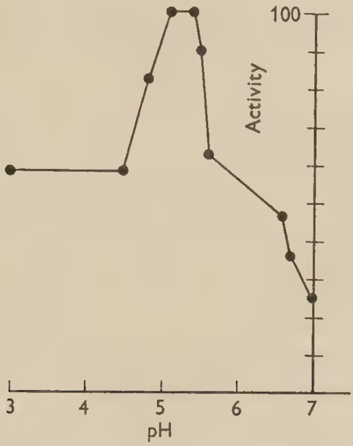

Fig. 1. Effect of pH value on *Botrytis cinerea* pectinase activity.

II. EFFECT OF CULTURAL CONDITIONS ON THE PRODUCTION OF PECTINASE BY *PYTHIUM DEBARYANUM*

Mycelial growth and pectinase production on natural decoctions

(1) Potato juice was prepared, autoclaved, the precipitate removed, and the clear liquid again autoclaved. The fungus grew moderately well on this medium but no pectinase capable of macerating potato disks within 24 hr. was obtained.

(2) Potato juice was autoclaved and both clear liquid and precipitate were incorporated in the medium. Some enzymic activity was shown but the effect was weak and irregular (maceration time 6–24 hr.).

(3) A potato decoction (D) was prepared by boiling 200 g. of potatoes in 200 ml. water for 1 hr. The liquid was squeezed through muslin and the thick liquid was used as such or in various dilutions. The results are shown in Table 6.

Table 6. *Pectinase production by* Pythium debaryanum *on potato decoctions*

Medium	Initia pH	Final pH	Dry wt. mycelium (mg.)	Time for maceration (hr.)
Potato decoction D	5·4	7·2	320	24
D/2	5·6	7·6	130	3
D/5	5·9	7·7	30	2–5

It was noted that the more dilute media gave the more active preparations. A further experiment showed that the comparative lack of activity in the richer medium was due to enzyme-retarding substances.

(4) Turnip decoction was prepared by boiling 200 g. turnips in 200 ml. of water. The decoction was used undiluted and in various dilutions but no enzyme production was noted.

Mycelial growth and pectinase enzyme production on synthetic media

Standard medium

The essential compounds contained in 100 g. of dried potato, as given by Thorp's *Dictionary of Chemistry*, were dissolved in 200 ml. of distilled water to give the Medium N: peptone 4·5 g., asparagin 4·5 g., glucose 5·0 g., starch 25·0 g., $MgSO_4.7H_2O$ 1·9 g., K_3PO_4 3·4 g., distilled water 1000 ml. This medium gives good growth and secretion of pectinase enzyme, and throughout the work with *P. debaryanum* the activity of the enzyme secreted in this medium was taken as 100.

Effect of elimination of various constituents of the standard medium

The effect of omitting each constituent or several constituents of Medium N on growth and pectinase activity is shown in Table 7.

Table 7. *Effect of constituents of Medium N on pectinase production by* Pythium debaryanum

	Initial pH	Final pH	Dry wt. mycelium (mg.)	Activity
Complete medium N	7·6	7·5	310	100
– Glucose	7·9	7·9	240	86
– Starch	7·8	8·0	60	70
– Starch and glucose	7·9	8·0	20	4
– Asparagin	7·6	7·5	400	4
– Peptone	7·6	7·4	330	14
– Peptone and asparagin	7·0	6·9	10	4
– $MgSO_4$	7·7	7·8	100	4
– K_3PO_4	6·7	6	160	4

It is shown from Table 7 that removal of any constituent of Medium N lessens activity, the least effective being glucose and starch. Removal of the last two and especially starch, reduces mycelial growth, so that the reductions in enzymic activity may be attributed to reduced growth. On the other hand, removal of either nitrogen constituent does not reduce growth, but reduces enzymic activity to a low value. Removal of glucose and starch or asparagin and peptone or of the mineral salts seriously reduces both growth and enzymic activity.

Effect of varying carbon and nitrogen sources on pectinase production

Keeping the starch and glucose constituents of Medium N constant, the concentrations of asparagin and peptone were increased and decreased (see Table 8). The variation in enzymic activity is very striking, the greatest activity being in the normal Medium N and dropping sharply with reduction or concentration of the nitrogenous constituents. The fall in enzymic activity which is shown at concentrations of nitrogenous constituents greater than normal was not expected and experiments were carried out to examine this further. Part of the effect at least was shown to be due to a retarding effect of excess asparagin or peptone upon enzymic activity.

Table 8. *Effect of nitrogen concentration on production of pectinase by* Pythium debaryanum

Medium N. Asparagin and peptone concentration	Initial pH	Final pH	Mycelium dry wt. (mg.)	Activity
N/10	7·4	6·9	220	2
N/5	7·5	7·45	340	2
N/2	7·5	7·6	360	11
N	7·5	7·7	330	100
2 N	7·2	7·55	240	8
4 N	7·0	7·4	260	2

A similar series of experiments was carried out in which asparagin and peptone were kept constant and the concentrations of glucose and starch varied upwards and downwards from the normal (see Table 9). The sharp fall in activity from N to N/10 is attributed to reduced growth. The drop in activity from N to 4 N runs counter to increased growth and is attributed partly to the unfavourable pH. Subsidiary experiments showed that very little of the reduced activity shown in media 2 N and 4 N is due to a retarding action of the concentrations of glucose and starch used.

Table 9. *Effect of carbohydrate concentration on pectinase production by* Pythium debaryanum

Medium N. Glucose and starch concentration	Initial pH	Final pH	Mycelium dry wt. (mg.)	Activity
N/10	7·9	8·2	70	8
N/5	7·9	8·05	90	8
N/2	7·8	7·9	150	20
N	7·6	7·45	290	100
2 N	7·4	6·2	660	14
4 N	7·2	5·9	790	10

Effect of varied concentrations of magnesium sulphate on pectinase production

Table 10 gives the effect of $MgSO_4$ varied between the limits 0–0·76 in a medium otherwise as Medium N.

Table 10. *Effect of* $MgSO_4$ *concentration on pectinase production by* Pythium debaryanum

Medium N. concentration of $MgSO_4 \cdot 7H_2O$	Initial pH	Final pH	Mycelium dry wt. (mg.)	Activity
0	7·7	7·8	50	5
0·005 %	7·6	7·5	170	5
0·01 %	7·6	7·6	230	5
0·02 %	7·8	7·7	300	220
0·04 %	7·8	7·8	230	135
0·1 %	7·8	8·2	190	100
0·19% (Medium=N)	7·6	7·6	290	100
0·38 %	7·4	7·7	310	68
0·76 %	7·3	7·4	300	22

Enzymic activity is high over the range 0·02–0·19 and is greater whe $MgSO_4$ concentration is lowered to 1/10 or 1/5 of the normal amour than it is in the standard medium. The highest concentration of MgSC used had a pronounced retarding effect on enzymic activity.

A similar experiment was carried out using various amounts of K_3PO_4. It was shown that enzymic activity was less on the standard medium tha when K_3PO_4 concentration is lowered to 0·5 or 0·2 of the normal amoun The activity is very low when higher concentrations of K_3PO_4 are use and this may be partly due to negligible growth.

Effect of dilution of Medium N

The effect of dilution or concentration of all the constituents of Medium N over the range N/20 to 2·5N is shown in Table 11.

Table 11. *Effect of dilution of Medium N on pectinase production by* Pythium debaryanum

Medium N	Initial pH	Final pH	Mycelium dry wt. (mg.)	Activity
N/20	7·4	7·4	20	20
N/10	7·5	7·5	30	75
N/5	7·6	7·7	70	175
N/2	7·6	8·0	120	215
N	7·6	7·8	310	100
2N	7·4	7·3	570	22
2·5N	7·4	6·8	680	18

It is clear that the maximum activity is shown in the more dilute media There is also a sharp drop in activity above the concentration N. Subsidiary experiments showed that Medium N exerted a retarding effect on media N/5 and N/2 and that the more concentrated media showed the same effect on Medium N itself.

Effect of pH *value on pectinase activity.*

A range of pH values was established by the addition of N/10-NaOH and N/10-HCl to equal volumes of the enzyme secreted in Medium N. The activity of each enzyme solution was estimated and compared with that of Medium N which was taken as 100 (see Fig. 2).

III. EFFECT OF PECTIN ON PECTINASE SECRETION BY *BOTRYTIS CINEREA* AND *PYTHIUM DEBARYANUM*

Harter & Weimer (1923) claimed that the secretion of pectinase enzyme by *Rhizopus tritici*, which takes place in certain plant juices but not in some synthetic media used, was stimulated by the presence in the latter of 1 % pectin. A study was therefore made of the effects of pectin prepared from lemon on pectinase secretion by *Botrytis cinerea* and *Pythium debaryanum*.

Botrytis cinerea. The effect of pectin on the production of pectinase by *B. cinerea* was investigated by replacing or supplementing the glucose in Medium A with pectin and by a combination of various concentrations of glucose and pectin in a medium having asparagin and mineral salts as in

Medium A. The results, which are summarized in Tables 12–14, show that with pectin as the sole carbohydrate source exoenzyme production increases with pectin concentration and reaches the same level as that in Medium A, although the amount of growth is much less (Table 12). The addition of pectin to Medium A has little effect (Table 13) on pectinase production but a decrease in the glucose/pectin ratio resulted in an increase of pectinase per mg. of dry weight of the mycelium produced (Table 14).

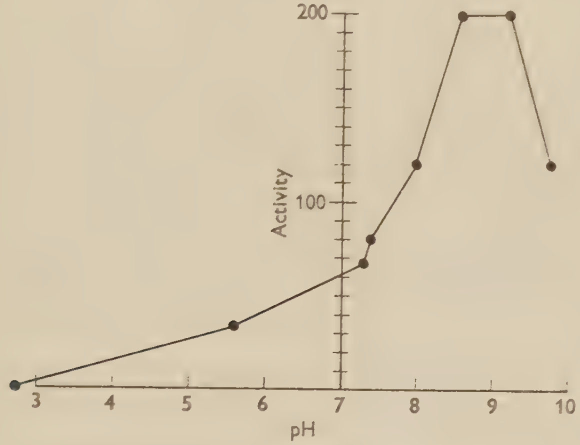

Fig. 2. Effect of pH on *Pythium debaryanum* pectinase activity.

Table 12. *The effect on pectinase production by* Botrytis cinerea *of replacing glucose in Medium A by pectin*

Glucose replaced by	Initial pH	Final pH	Mycelium dry wt. (mg.)	Activity
0·1 % pectin	4·2	7·3	24	16
0·25 % pectin	4·0	7·1	40	33
0·5 % pectin	3·6	7·3	77	40
1 % pectin	—	6·8	104	100
2 % pectin	2·8	6·2	185	73
Medium A	4·5	5·5	370	100

Table 13. *The effects on pectinase production by* Botrytis cinerea *of additions of pectin to Medium A*

Medium	Initial pH	Final pH	Dry wt. mycelium (mg.)	Exoenzyme activity	Endoenzyme activity
A	4·5	5·6	330	100	100
A+0·25 % pectin	4·0	6·3	310	140	90
A+0·5 % pectin	3·8	4·9	270	155	100
A+1 % pectin	2·9	6·6	330	210	90
A+2 % pectin	2·8	2·8	310	210	125

Pythium debaryanum. For *P. debaryanum* pectin was either substituted for or used to supplement the carbon source in five media: Brown's Medium A, Brown's Medium B, modified Czapek's medium, Richard's medium, and

Table 14. *The effect of the glucose/pectin ratio on pectinase production by* Botrytis cinerea

Media: asparagin and salts as Medium A with					
Glucose (%)	Pectin (%)	Initial pH	Final pH	Dry wt. mycelium (mg.)	Activity
2·5	0	4·5	5·8	450	100
0·625	1·875	3·9	6·8	340	120
1·25	1·25	3·3	7·0	300	110
1·875	0·625	3·0	6·8	220	110

Medium N. Only in certain modifications of Medium N was there any appreciable enzyme production and in none was the enzyme production as good as that in Medium N unsupplemented by pectin. These results suggested that *P. debaryanum* is unable to utilize pectin as a carbon source and that pectin may even be detrimental to pectinase production by that fungus. The non-utilization of pectin by *P. debaryanum* was confirmed by determining by Haynes and Carré's method (as described in Norman, 1929) the amount of pectin present in a synthetic medium 4, 8 and 17 days after inoculation.

The writer is highly indebted to Prof. W. Brown, F.R.S., of the Imperial College of Science and Technology, London, for suggesting the problem and for his inspiring guidance and criticism.

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A STUDY OF PHYSIOLOGIC SPECIALIZATION OF THE FLAX RUST, *MELAMPSORA LINI*[*]

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From thirty-eight collections of *Melampsora lini* (Ehrenb.) Lév. from crops grown in Northern Ireland, England, Scotland, Wales and Pakistan, seventeen physiologic races have been distinguished by the reaction of seventeen differential flax varieties including nine new races designated by the letters A to J. No difference in susceptibility or resistance was found when plants were inoculated at the ages of 10 or 30 days. Some of the physiologic races are fairly widely distributed.

When tested under greenhouse conditions all the fibre varieties of flax commonly grown in the United Kingdom were susceptible to all the races isolated during the investigation. Certain imported varieties of fibre flax, including Textilschchik, Stakhanovets, Wada, 1288/12 and U.S.S.R. no. 2 (ex Dugejheshchya), were immune or highly resistant to all races in greenhouse tests.

Varieties susceptible in greenhouse tests were also susceptible in the field when inoculated with the same races at various stages before flowering, but were resistant if inoculated after flowering.

INTRODUCTION

It was demonstrated almost 90 years ago that the race of *Melampsora lini* (Ehrenb.) Lév. attacking cultivated flax (*Linum usitatissimum* L.) was physiologically distinct from that causing rust of certain wild species of *Linum*. Flor (1935), working in North America, demonstrated for the first time that different physiologic races of the pathogen occurred on cultivated varieties of flax. Such specialization was later confirmed by other workers elsewhere (Straib, 1939*a*, *b*; Vallega, 1938, 1944; Padwick, 1940; Waterhouse & Watson, 1941, 1944). More recently, Flor (1946) has demonstrated that new races of the fungus can be produced by crossing known races.

Flax seed has been imported into the British Isles from many countries over a long period. It would appear probable that many races of *M. lini* might have been introduced with imported seed or have arisen at a later date within the country by the crossing of races. It therefore appeared desirable to investigate whether or not physiologic specialization of the parasite occurs in this country, and if it does occur to ascertain to what extent the races present are comparable with those existing elsewhere.

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MATERIAL AND METHODS

Uredospores of the fungus were collected in 1947 from a number of crops in Northern Ireland. Many collections were from the lands of the Plant Breeding Division, Stormont, and the Agricultural Research Institute, Hillsborough, where large numbers of oil and fibre varieties were growing, but collections were also made from farmers' crops. Six collections from different parts of Great Britain and one from Pakistan were obtained through the co-operation of a number of plant pathologists. From each collection a single-spore culture was made and maintained in a rust-proof chamber on plants of variety Stormont Gossamer for use in experimental work.

For separating physiologic races Flor (1946) used sixteen differential varieties. Working in Germany, Straib (1939*b*) had found it necessary to include the variety Redwing which was not employed by Flor. These seventeen varieties were used in the present work. Seed of all varieties except Redwing (C.I. no. 320) was kindly supplied by Dr Flor and so is comparable with that used by him and other workers.

When differentiating physiologic races the inoculation methods employed by Flor (1935) and Straib (1939*a*) differed in that Flor inoculated 30-day-old plants taking into consideration buds, leaves and cotyledons, whereas Straib inoculated only the cotyledons of 7–10-day-old seedlings. In the present work both these methods were followed to determine if any difference exists between the susceptibility or resistance of plants at the two stages of growth. After inoculation the plants were kept in a moist atmosphere in rust-proof chambers at a temperature of 13–15° C. for 36 hr. and then removed to rust-proof chambers in a greenhouse at a temperature of 13–20° C. Each set of plants inoculated with a monospore culture was kept in a separate chamber.

Each of the rust-proof chambers employed in the greenhouse consisted of a wooden frame measuring 75 × 60 × 45 cm. The sides were of 'Windolite' and the roof, back and front, of 'Cellophane'. The front of the chamber could be readily removed and replaced to permit of easy access to the plants with the minimum of exposure. During the latter part of the work 'Perspex' was satisfactorily substituted for 'Cellophane' in the construction.

The plants inoculated in greenhouse experiments were grown in 4 in. pots filled with good loam. Normally ten plants were grown in each pot. Propagation was carried out in a greenhouse, free from rust spores, at a temperature of 15–20° C.

Since light is essential not only for good growth of the host plants but for the development of uredosori, the greenhouses were fitted with 200 W. electric lamps to provide a light day of 16 hr. during winter.

Uredosori usually appear 8–12 days after inoculation, and observations were made 11–14 days from the time when the plants were inoculated. The type of infection produced on the differential varieties enabled the physiologic races to be distinguished. Infection type may be regarded as the visible expression of the reaction between the host and parasite or as a measure either of the susceptibility of the host or of the pathogenicity of the parasite. The classes of host reaction followed were based on the types of uredosori as set out by Flor (1935) and Straib (1939*a*). Three classes were recognized, the appropriate abbreviation used later for each being as follows: immune (I); resistant (R) and susceptible (S). Plus and minus signs indicate more or less resistance or susceptibility than the letter indicating the reaction.

When making observations on the types of infection on the inoculated plants minor differences in the reactions to different cultures were disregarded. Tests with different cultures were repeated two or three times, and when any doubt arose as to the reaction of the differential varieties to a culture, the tests were repeated until three results agreed. At least one test with each culture was made during spring or summer. Normally ten plants of each differential variety were inoculated with the culture in each test.

RESULTS

Differentiation of physiologic races

The seventeen differential varieties were inoculated with thirty-eight cultures, observations being made on the infection types shown by the varieties to each culture when 30-day-old plants (Flor's technique) and 10-day-old plants (Straib's technique) were used. No difficulty was experienced in obtaining infection of the different varieties. No dissimilarity in reaction of the differential varieties to the various cultures was observed when the techniques of Flor and Straib were employed, the varieties which were immune, resistant or susceptible according to one technique showing a similar reaction when inoculated according to the other. As a result of this work seventeen physiologic races of the fungus were distinguished, the reaction of the differential varieties to each being stated in Table 1.

Table 1 shows that eight of the races have already been described by Flor (1935) or Straib (1939*a*, *b*, 1943). Three of these are comparable with Straib's races (Straib, 1939*b*), H-1 (recorded from Holland), S-3 (recorded from Sweden) and D-2 (recorded from Germany), to which Flor (1942) has assigned the race numbers of 25, 29 and 30. Two can be referred to races D-5 and D-6 isolated from material collected in Germany by Straib (1943). Three others correspond with Flor's races 1, 4 and 5 (recorded from North America), except that Pale Blue Crimped is now recorded as showing rather more resistance to each of them. Such differences can, however, be disregarded, for in consequence of the reaction of Pale Blue Crimped to avirulent cultures being influenced by environment and varying from resistant to moderately susceptible it was classed as resistant to all avirulent cultures (Flor, 1946). Neither Flor nor Straib at the time they differentiated the above-mentioned races, employed as many differential varieties as were used in this investigation. The reactions to races 1, 4 and 5 given in Table 1 for those varieties not employed by Flor in 1935, as well as the reactions of Pale Blue Crimped, agree with those stated by him in a private communication.

Table 1 shows also that nine of the races differentiated have not been referred to any described previously and are distinguished by the letters A, B, C, D, E, F, G, H and J; J shows considerable similarity to New Zealand race 9 recently isolated by Cruickshank (1952).

Table 1. *Reaction of differential varieties to cultures of* Melampsora lini

Culture no.	Buda	Williston Golden	Williston Brown	Akmolinsk	J.W.S.	Pale Blue Crimped	Kenya	Abyssinian	Morye	Ottawa 770B	Bombay	Newland	Bolley Golden	Italia Roma	Leona	Tammes Pale Blue	Redwing	Race no.
1, 13, 25, 75	R	I	S	I	I	R−	I	I	I	I	I	I	I	I	I	I	R	Race 1 (Flor)
19, 52, 65	S	I	S	R	I	R	R+	I	I	I	I	I	I	I	I	I	S	Race 4 (Flor)
53	R	R+	S	S	I	R	R+	R	I	I	I	I	I	I	I	I	S+	Race 5 (Flor)
12, 21, 24, 29, 42, 47, 51, 63	S+	I	S+	S+	I	R	I	S+	I	I	I	I	I	I	I	I	R	Race H-1 (Straib) = Race 25 (Flor)
14, 18, 32	S	I	S+	S+	I	R	I	S+	I	I	I	I	I	I	I	I	S	Race S-3 (Straib) = Race 29 (Flor)
26	R+	R	S+	R	S+	R	I	R	I	I	I	I	I	I	I	I	S+	Race D-2 (Straib) = Race 30 (Flor)
17	S+	S	I	R	I	I	I	I	I	I	I	I	I	I	I	I	R	Race A
27	S+	I	S	R	I	S+	R	I	I	I	I	I	I	I	I	I	S+	Race B
5, 15, 28, 74	R	R	S	S	I	R	R	S+	I	I	I	I	I	I	I	I	R	Race C
50, 62	R	R	S	S	I	R	R	S+	I	I	I	I	I	I	I	S	S	Race D
11, 56	R	R	S	S	I	S+	R	S+	I	I	I	I	I	I	I	I	S	Race E
6	S	I	S	S	S	S	I	S	I	I	I	I	I	I	I	I	S	Race D-6 (Straib)
31	S+	I	S+	S+	I	S+	I	S+	I	I	I	I	I	I	I	I	R	Race F
76	R+	S	S	S	S	I	I	I	I	I	S	I	I	I	I	I	R+	Race G
10, 36, 67	S+	I	I	S	I	R	I	S+	I	I	I	I	I	I	I	I	R+	Race H
9	R+	I	S	S	S	R+	I	S	I	I	I	I	I	I	I	I	S	Race D-5 (Straib)
37	S	R+	S	S	I	S	R+	R+	I	I	I	I	I	I	I	I	R	Race J

Variability in reaction of flax varieties used as differential hosts

Flor (1940) has pointed out that some differential varieties may show rather poor stability in their general reaction under varying conditions and therefore exhibit a wide reaction range with certain physiologic races. Since the reaction of such varieties was influenced by changes in environmental conditions, considerable care was necessary in differentiating between degrees of resistance or susceptibility. During the present investigation such variations were observed, but when the cotyledons of 10-day-old plants were inoculated they were comparatively slight as compared with those arising from the inoculation of 30-day-old plants. It was also seen that the infection and symptoms of the disease were clearer when Straib's technique was employed. Some varieties, including Bombay, Ottawa 770B, J.W.S., Leona, Tammes Pale Blue, Bolley Golden, Italia Roma, Newland and Morye, showed no variation. These varieties showed uniform immunity or susceptibility under all conditions of inoculation to the physiologic races to which they were immune or susceptible and are therefore ideal differentials. Although, as reported by Flor (1940), Williston Golden and Abyssinian showed slight variation, no difficulty was experienced in classifying their reactions. On the other hand, to some of the physiologic races, Williston Brown and Akmolinsk showed more variation than the above-mentioned varieties, but the reaction was never confusing. The varieties Buda, Pale Blue Crimped and Redwing were sensitive to changes in environmental conditions, and their reactions were sometimes difficult to classify. To some races, Buda was so stable that even under changing conditions its reaction was very clear, but to other races it showed much instability. In general, its reaction can be regarded as stable under optimum conditions for plant growth. The variety Redwing behaved similarly. The reaction of Pale Blue Crimped showed much variation, especially with varying growth conditions. Under favourable conditions for growth it became more resistant to races to which it had otherwise shown a measure of susceptibility, this being in agreement with the finding of Flor (1940). The considerable variation of the variety Kenya observed by Flor was not noticed.

Geographical distribution of physiologic races

The number of cultures studied was limited to thirty-eight, and although these cannot be considered as providing complete data on the distribution of races they produce some interesting information.

All the races except G and J occurred in Northern Ireland. Race G occurred only in a crop grown in Pakistan and race J in an English crop. The other culture isolated from English crops was assigned to race H. The only culture originating from a crop in Scotland was assigned to race 1. Three cultures from Welsh crops were studied and referred to races 1, 25 and D.

Race 25 was isolated on eight occasions and appeared to be the most common. Three cultures obtained from each of two localities in Northern Ireland were assigned to this race which also occurred at a third locality in Northern Ireland and in Wales. Races 1 and C were isolated on four occasions, races 4, 29 and H on three occasions, while races D and E were isolated twice. All the other races were isolated once only.

Reaction of commercial flax varieties

Having separated the physiologic races they were then used to determine the relative resistance and susceptibility of numerous commercial varieties of flax. Approximately twenty each of 30-day-old and 10-day-old plants of each variety were inoculated with each race, and when there was any doubt as to the reaction of a variety the test was repeated. In certain varieties where a uniform reaction was not obtained the predominating reaction was recorded.

The following varieties were susceptible to all the physiologic races: Danish 40, Linkopis, Liral Prince, Liral Dominion, Liral Crown, Liral Monarch, Stormont Motley and U.S.S.R. no. 1 (ex Shakhunskaya). Varieties which were susceptible to all races, except race G, to which they were immune or resistant, included Bison, Blenda, Concurrent, Curitiba, Giza Purple, Hunter's Hybrid, Lyngby 7, Norfolk Queen, Pinnacle, Stormont Cirrus, Stormont Gossamer and Saginaw. Formosa was resistant or immune to all races except G. Hollandia and Punjab (Type 15) were susceptible to G but immune to the others, while Winona showed either resistance or immunity to all. Stakhanovets, 1288/12, Wada and U.S.S.R. no. 2 (ex Dugejheshchya) showed resistance to G and were immune to all others. The only varieties showing immunity to all races were Koto and Textilschchik.

Field experiments

The experiments already described were made in a greenhouse, and the question arises whether the results obtained can be applied to the reaction of older plants in the field. A field experiment was accordingly made using four flax varieties and four physiologic races of the fungus. The varieties used were Williston Brown, Buda, Abyssinian and Morye, while the physiologic races employed were 1, 4, 25 and D. Ten plants of each variety were inoculated with each race at the desired stage of growth, the method of inoculation being to dust the spores on to the plants with a brush. A further ten plants of each variety in each of two plots were inoculated with each race at the appropriate stage of growth, but these inoculations were made by spraying the plants with a suspension of uredospores in 0·1 % agar. The plants were inoculated at the following ages or growth stages: (*a*) 7–10 days, (*b*) 30 days, (*c*) before flowering, and (*d*) after flowering. All the experimental plants were covered with 'Polythene' bags during the entire period of growth before and after inoculation. The results obtained for the reaction of plants are presented in Table 2.

Table 2 shows that no infection occurred when plants were inoculated after flowering. When inoculated at any of the three earlier stages they showed reactions comparable with those observed in greenhouse studies, although the intensity of infection was low.

In this experiment heavier infection resulted when plants were dusted with spores than when they were sprayed with a spore suspension.

Table 2. *Results of inoculations made in the field*

Variety	Age or growth stage at which plants were inoculated	Race numbers 1	4	25	D
Buda	7–10 days	R	S	S	I
	30 days	I	S	S	I
	Before flowering	R	S	S	I
	After flowering	I	I	I	I
Williston Brown	7–10 days	S	S	S	S
	30 days	S	S	S	S
	Before flowering	S	S	S	S
	After flowering	I	I	I	I
Abyssinian	7–10 days	I	I	S	S
	30 days	I	I	S	S
	Before flowering	I	I	S	S
	After flowering	I	I	I	I
Morye	7–10 days	I	I	I	I
	30 days	I	I	I	I
	Before flowering	I	I	I	I
	After flowering	I	I	I	I

DISCUSSION

The results presented show that sixteen physiologic races of *M. lini* have been distinguished as occurring on flax grown in the United Kingdom. Among these are a few North American and European races as well as a number not hitherto described. The occurrence in this country of the North American and European races may be attributed to the importation of large quantities of flax seed from Canada and the continent of Europe over long periods.

Of the physiologic races now described as new, four are distinguished from races previously described solely on the reaction of the differential variety Pale Blue Crimped. This variety was very susceptible to three of these four races and susceptible to the remaining one. Flor (1940) observed that Pale Blue Crimped showed a more resistant reaction under normal growth conditions as compared with unfavourable conditions, and his finding has been confirmed during the present work. It is, however, believed that the reactions of this variety to the cultures assigned to these new races were sufficiently definite under the different conditions employed to justify their differentiation.

Flor (1935, 1940) and Straib (1939*a*, *b*) employed different techniques for the identification of races of *M. lini* and thus a direct comparison between their work was not possible. Straib observed that infection of cotyledons was more severe and regular than that of foliage leaves, and that the results of cotyledon infection corresponded to those on foliage leaves. In the present work, where the techniques of Flor and Straib were used, no difference was observed in the susceptibility or resistance of plants at the two different stages of growth employed. However, the method of infecting cotyledons is preferred as time is saved, and also because the varieties do not give much variation with changes in environmental conditions.

Vallega (1938) observed in field experiments that attacks of rust were more severe before flowering and decreased when the flowering period was over. It is now shown that, although plants are equally susceptible when inoculated at any stage before flowering, yet infection does not result when inoculations are made after flowering. The results presented also show that plants of varieties which were susceptible when inoculated in the greenhouse at 10 or 30 days old were also susceptible in the field when inoculations were made at any stage before flowering.

The resistance of a number of flax varieties commonly grown in the United Kingdom and a few of foreign origin was tested in the greenhouse to all the physiologic races of *M. lini* differentiated during the work. No variety of fibre flax commonly grown in the United Kingdom was found to be resistant to any of the physiologic races identified from that area. Textilschchik was immune to all races studied. A small number of other fibre varieties, not of British origin, Stakhanovets, Wada, 1288/12, U.S.S.R. no. 2 (ex Dugejheshchya) and Winona, were immune or resistant to all British races isolated. The oil variety, Koto, was also immune to all races, while the fibre varieties, Formosa and Hollandia, were resistant or immune to all the British races although susceptible to race G, recorded from Pakistan. When these results are compared with those obtained in Northern Ireland by Colhoun (1948) under field conditions where the physiologic races were not controlled, it is found that the results agree except that Textilschchik was not quite immune and Winona, Hollandia and 1288/12 were susceptible in the field, although they showed resistance to all the United Kingdom races in the greenhouse. The disparity of the results in respect of these varieties could be explained by the fact that more physiologic races were presumably present in the field tests. Eight of the cultures now studied were taken from the field where these varieties were attacked and six distinct races were isolated. If more cultures, including some from the pathogen on these four varieties, had been studied it should have been possible to isolate races which would have attacked them.

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ON THE BIOLOGY OF *BREMIA LACTUCAE*

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(With 3 Text-figures)

Heavy infection of lettuce seedlings by *Bremia lactucae* spores was induced by 24 hr. incubation (minimum period may be as low as 4 hr.) in a saturated atmosphere; this was followed by 7–10 days under ordinary conditions (i.e. humidity not controlled), and finally by 1–2 days' exposure to high humidity to encourage sporulation.

Infection following different temperature treatments during the first moist period was heavy over the range 2–20° C., but did not occur at 25° C. Temperatures in the range 4–20° C. during the second moist period allowed of sporulation, but none occurred at 1–2° C. or at 25° C. The relative humidity necessary for sporulation ranged from 100% to between 80 and 90%.

Good spore germination occurred in tap water, and the percentage of spores which germinated was not increased by the addition of certain nutrients.

Optimum temperature for germination was in the region of 10° C., although at 15° C. germination was more rapid and the germ-tubes grew more quickly in the initial stages.

Bremia spores are short-lived, showing a much reduced germinating capacity even after 3 days. Viability was greatest for spores kept at relative humidities above 50% but was generally lost after the eighth or ninth day, whereas below 50% relative humidity viability was lost after 2 days.

Microscopic examination of inoculated leaves showed that penetration had taken place over the temperature range 1–20° C., but not at 25° C. Penetration was cuticular and in no instance was stomatal penetration observed.

Cross-inoculation experiments confirmed the results of previous workers showing the existence of biological races of *B. lactucae*.

INTRODUCTION

Although lettuce downy mildew has been known for many years, the literature relating to its causal fungus *Bremia lactucae* Regel is not extensive and is in part contradictory. Schweizer (1919), in a study of the factors controlling spore germination, states that at low temperatures germination was strongly or completely repressed. This was not confirmed by Schulz (1937), who found that good germination took place even at a temperature as low as 1–2° C. Infection occurred over the range 2–19° C. and spores could be produced over the same range, but not at 24° C. *Bremia* was stated by Milbraith (1923) to remain vigorous and even to flourish on lettuces in refrigerator cars. He also reported that spores of the fungus sometimes behaved as zoosporangia, especially when kept in darkness. Most if not all other workers have reported the tube method of germination. The important bearing of temperature and atmospheric humidity on development of the disease was stressed by Schulz & Röder (1938) who stated that no new lesions developed on plants in the open during a hot and almost rainless period, whereas spores were immediately produced after a thunderstorm. This was cited as evidence that the fungus had gained a foothold at an earlier stage but that it was unable to sporulate until there was a high atmospheric humidity.

In the course of experiments on the fungicidal control of lettuce mildew, large-scale inoculation of lettuce seedlings in frames was necessary as natural infection, which fluctuates considerably from year to year, could not be relied upon. This involved the preparation of spore suspensions in quantity whenever required. It was incidental to this routine that the factors which affect spore germination, spore viability, infection, etc., were worked out in some detail. The following paper gives an account of these investigations.

MATERIALS AND METHODS

The strain of *B. lactucae* used was obtained from outdoor lettuces growing at Harlington, Middlesex, in September 1950. This was inoculated on to seedling lettuces growing in seed-boxes in the greenhouse. The method of maintaining stock cultures was as follows.

About 7–10 days after sowing, the lettuce seedlings, which at this stage were only showing their cotyledons, were sprayed with a spore suspension. The plants were sprayed until the leaves were completely wet, then immediately placed in a moist chamber in the greenhouse (15–21° C.). After 24 hr. of such treatment, the boxes were transferred to a cold frame where they were kept under ordinary conditions until needed, i.e. no attempt was made to control the humidity. A period of 7–10 days was necessary for the fungus to spore, and it would then do so only on being kept overnight in a saturated atmosphere. Infected, but non-sporulating, plants of the size used could be made to produce a crop of spores up to about 3 weeks from the time of inoculation. If kept for a longer period, the plants had generally grown fairly large and the infected cotyledons and older leaves had frequently shrivelled up. It was a routine procedure to sow ten or twelve seed boxes with lettuce every 10 days and to inoculate 7–10 days later.

The lettuce variety used throughout was Trocadero Improved (white seeded). This is the variety of spring lettuce most commonly grown in the Thames Valley district, and it is highly susceptible to *Bremia*. Under the conditions adopted, 70–100% of the sprayed plants regularly showed infection.

For purposes of inoculation studies, plants were grown in cylindrical tins (7·5 cm. diam., 7·5 cm. deep), protected from rust by a thin film of bituminous paint and with a few holes punched in the bottom. About twenty seedlings were grown in each of these.

Germination studies were carried out with drops of standard size (0·03 ml.), and the spore concentration was adjusted to give approximately 20 spores per microscope field ($\frac{1}{3}$ in. objective, × 20 ocular). As examining the drops to determine the stage of germination was a lengthy process, it was important to stop germination and further growth at the same time. This was done by adding a little iodine in potassium iodide to each germination drop, and the measurements could then be carried out at leisure. In these experiments on germination, the operation of setting up the slides was done as quickly as possible, always within 30 min. As a standard procedure 100 spores in each drop were counted. The average germ-tube length was also determined in some experiments by making twenty measurements in each drop. Incubation was carried out in Petri-dish moist chambers in which 'clarity' paper was used to maintain a saturated atmosphere.

CONDITIONS GOVERNING INFECTION AND SPORE FORMATION

Length of period of high humidity following inoculation

The period of high humidity following inoculation is required to allow of spore germination and entry of germ tubes into the plant. It was known that 24 hr. was adequate for the purpose, but a more accurate determination was considered desirable and this was obtained by the type of experiment summarized in Table 1.

Table 1. *Determination of minimum incubation period for establishment of attack by* Bremia *spores at* 15–21° C.

Duration of wet period (hr.)	% germination on slide	Av. length of germ tube (μ)	% plants infected: Sprayed with Dithane	% plants infected: Dried in air
1	0	0	0	28
2	4	13	0	26·5
3	8·8	37	0	34
4	14·8	61	11·5	47
6	10·3	100	49	86
10	27	101	85	100
16	61	150	93	100
24	90	190	99	97

The unit for each treatment was five tins, each containing about twenty seedlings. Altogether 180 such tins were used. These were placed, after being sprayed with a suspension of *Bremia* spores, in a saturated atmosphere, and at intervals ten were removed. Of these five were immediately placed in a draught of warm air so as to dry up the drop of inoculum as quickly as possible, a treatment which would, it was thought, stop further germination and growth of spores which had not already entered the host tissue. As an alternative method, the other five were sprayed with Dithane (zinc ethylene bisdithiocarbamate), 4 g./l., which is actively fungicidal. After these treatments the plants were placed in a cold frame for 10 days and then in a moist chamber for 2 days to allow of sporulation where infection had taken place. A parallel series of germination measurements was carried out on glass circles in the laboratory.

In the series where Dithane was used, infection had not occurred after 3 hr., but after that time it progressively increased and by 10 hr. had reached a value of approximately 80–90 %. The corresponding figures for the plants which were dried in warm air were distinctly greater, for initial moist periods not exceeding 10 hr., than those of the Dithane series, and in particular there was appreciable infection even after only 1 hr. sojourn in the moist chamber. At that time germination had not begun and the conclusion obviously is that the drying process was not effective in stopping spore germination and growth quickly enough; the results of the Dithane series are therefore the ones which are relevant.

This experiment was repeated twice with substantially the same result, viz. infection began sporadically after 2–3 hr. and reached a high value after 10–12 hr. It was clear therefore that a period of 24 hr. exposure to high humidity was ample for heavy infection under ordinary greenhouse conditions (15–21° C.).

Effect of age of spores on germination and infection

Spore age can be controlled only in the sense that one can control the date at which sporulation begins. This is done simply by taking infected plants, after 7–10 days from inoculation, and placing them in a humid atmosphere. A crop of spores develops within 24 hr. If these spores are not used for 7 days, one can say that some of them—and probably the majority of them—are 6 days old. One cannot exclude the possibility that some are younger. With this reservation, one can speak of 1-day-old, 3-day-old and 6-day-old spores, as in Tables 2 and 3, the former of which relates to germination on glass and the latter to infection of seedlings. In Table 2 the temperature was 16·5° C., in Table 3, 15–21° C.

Table 2. *Percentage germination in water of spores of different ages (temp.* 16·5° C.)

Germination period (hr.)	Age of spores (days)		
	1	3	6
2	93	46	30
6	96	58	38
24		As after 6 hr.	

Even in spores 3 days old a distinct falling-off in germination capacity had occurred.

Table 3. *Percentage plants infected by spores of different ages (temp.* 15–21° C.)

Duration of incubation period at high humidity (hr.)	Age of spores (days)		
	1	3	6
2	8	6	1
3	4	7·5	0
4	9	8	0·7
5	16	9	5
8	40	33	33
24	93	86	85

These data of infection follow the same general course as those for germination, apart from some irregularity in detail. With short incubation periods there was an obvious falling off in establishment of disease as age of spore increased. Nevertheless, after 24 hr. incubation, approximately 100% infection was achieved by spores of all three ages tested. Germination of the older spores was slow, and after the shorter intervals few of them would have had time to penetrate. After longer periods of moist conditions there would be sufficient germination in any drop of inoculum for some germ-tubes to have penetrated the host tissues.

Effect of certain nutrients on germination

In preparing spore suspensions for germination studies conidia were brushed off from fresh mildew lesions with a wet camel-hair brush. For inoculation work where a large bulk of spore suspension was needed, this method was too slow and a different technique was adopted. Sporulating plants were cut off at soil level and put into a large glass beaker. A little water was added and the plants stirred vigorously to detach the spores from the leaves. This stirring caused some damage to the leaves which would lead to the escape of plant juices. The liquid was then filtered through fine muslin to remove conidiophores and dirt, and the resulting generally strong suspension of spores was diluted as required.

An experiment was set up to compare the percentage germination of spores collected in these two ways. To a third series of spores brushed off the leaves, a little macerated lettuce tissue was added.

After 24 hr. at laboratory temperature a germination of 80–90 % was seen in all the preparations, so that the presence of a small amount of lettuce extract had no material effect upon germination.

In his work on *Bremia*, Wild (1947) used tap (hard) water in preparing his spore suspensions. A detailed comparison by the writer of tap water, copper-distilled and glass-distilled water showed only small and irregular differences in their effect on spore germination.

Slight acidification, as in the presence of 0·001 % citric or malic acid, somewhat depressed germination as compared with that obtained in tap water. In 0·2 % sucrose solution germination was slightly less than in water alone. The effect of nutrients was not followed up in detail, but it did not seem that any advantage was to be obtained by adding anything to water as a medium for germination. In this respect *Bremia* conforms with obligate parasites in general.

Effect of temperature on germination, infection and sporulation

The effect of temperature on germination was studied over the range 1–25° C. with spores of three ages: 1, 3 and 6 days old. After 2 hr. incubation, a clear optimum was shown at 15° C., but 4 hr. later this had moved down to 10° C. After 24 hr. the percentage germination was not markedly different over the range 1–10° C. for all three ages of spore used. Above 15° C. for the older spores and above 20° C. for the younger ones it fell off sharply and was negligible for all at 25° C.

When the criterion of germinative vigour was average germ-tube length, the same general effect was obtained. The optimum in the early stages was near 20° C. but later fell back to about 10° C. Though the percentage of germinated spores after 24 hr. was unaltered between 1 and 10° C., the average germ-tube length at that time was progressively shorter the lower the temperature.

The effect of temperature on infection was closely parallel to that on germination. After a period of 24 hr. incubation in a moist atmosphere at a range of temperatures between 2 and 25° C., no difference was shown within the limits 2 and 20° C. (the percentage of infected plants ranging between 70 and 80). On the other hand, inoculation and moist incubation at 25° C. gave no infection.

The effect of temperature on induction of sporulation on infected plants was determined in two experiments. In each of these a batch of twenty-eight tins of seedlings was inoculated, exposed for 24 hr. to high humidity and then kept under ordinary conditions for 10 days. They were then transferred to moist chambers, consisting of large glass dishes containing water and covered with bell-jars, at temperatures ranging from 1 to 25° C. Four tins each containing about twenty seedlings were placed at each temperature. The results in Table 4 give the percentage of infected plants which produced spores.

Table 4. *Effect of temperature of second moist period on sporulation*

Temp. (° C.)	% sporulation Exp. 1	Exp. 2
1–2	0	0
4	40	53
6–11	96	71
9	91	94
15	91	95
20	96	92
25	0	0

Temperatures from 4 to 20° C. allowed of sporulation, but to a reduced extent at 4° C. No sporulation occurred at 1–2 or at 25° C.

B. lactucae spores are thus able to germinate and readily bring about attack, and in due course give rise to new crops of spores, at temperatures which are only a few degrees above the freezing point. Above 20° C. activity of the fungus in any form sharply falls off. These results are in close agreement with those of Schulz (1937).

Effect of relative humidity on sporulation

Differential relative humidity treatments during the second period of exposure to high humidity were carried out with infected seedlings which had been kept in the greenhouse under ordinary conditions for 10 days following inoculation. Each specimen tube contained one seedling growing in soil, the surface of which was sealed with 'Vaseline' just before putting the tubes into the controlled atmosphere. Each vessel contained a layer of the humidity-controlling liquid in which ten specimen tubes stood. The liquids used were: water (R.H. 100 %); H_2SO_4, sp.gr. 1·09 (R.H. 95 %), sp.gr. 1·14 (R.H. 90 %), sp.gr. 1·21 (R.H. 80 %) and saturated salt solutions of $(NH_4)_2SO_4$, $ZnSO_4 . 7H_2O$ and $Na_2SO_3 . 7H_2O$ giving relative humidities of 81, 90 and 95 %, respectively. The number of plants which produced spores is shown in Table 5.

The results shown in Table 5 are peculiar in that no sporulation occurred over H_2SO_4 at relative humidities of 90 and 95 %, whereas there was good sporulation at these humidity levels when obtained by use of saturated salt solutions. The results with H_2SO_4 are therefore open to question, and it is probable that the vapour pressure of the acid itself affected the results.

Inasmuch as a relative humidity of 81 %, as obtained by use of saturated ammonium sulphate solution, prevented sporulation, and as the salt is not volatile, one may accept this result as sound; the conclusion therefore is that the relative humidity necessary for sporulation ranges from 100 % down to a point between 80 and 90 % R.H.

Table 5. *Effect of relative humidity of air on sporulation*

Humidity-controlling agent	% R.H.	Plants sporulating
H_2SO_4, sp.gr. 1·21	80	0
H_2SO_4, sp.gr. 1·14	90	0
H_2SO_4, sp.gr. 1·09	95	0
$(NH_4)_2SO_4$, saturated solution	81	0
$ZnSO_4.7H_2O$, saturated solution	90	9
$Na_2SO_3.7H_2O$, saturated solution	95	9
Water	100	10

VIABILITY OF SPORES EXPOSED TO DRYING

Spores were exposed at 20° C. for periods up to 24 hr. in atmospheres of various relative humidities, a range of the latter being obtained by use of a number of saturated salt solutions (Table 6).

Table 6. *Effect of exposure to drying atmospheres on viability of spores*

		Period of exposure (hr.)		
Salt solution	% R.H.	2	6	24
Control = water	100	57	66	70
$Na_2SO_3.7H_2O$	95	56	41	37
$(NH_4)_2SO_4$	81	52	57	43
KNO_3	72·6	52	35	18
$NaHSO_4.H_2O$	52	46	31	15
$CaCl_2.6H_2O$	32·3	38	34	17

With the sample of spores used, germination was moderate in the controls, averaging about 65 %. After 24 hr. exposure to drying there was a falling off in viability at all humidities as expressed by the percentage of spores which germinated after incubation at 10° C. At the lower humidities and especially below 50 %, there was a drop in viability after only 2 hr. exposure, and this became very pronounced after 24 hr.

Experiments in which spores on detached leaves were exposed to prevailing atmospheric humidity and laboratory temperature (18° C.) showed that they were not viable after the 8th or 10th day. Even after 4 or 5 days, germination of the spores had fallen to the neighbourhood of 10 %. These observations are in good agreement with those of Schulz (1937) and Wild (1947) and emphasize the fact that spores of *Bremia* rapidly lose their power of spreading the disease unless a high atmospheric humidity is maintained.

MODE OF PENETRATION INTO HOST

Penetration of the host tissue by members of the Peronosporales appears to be usually by way of the stomata, though cuticular penetration is also recorded for the potato-blight fungus. Schulz (1937) figures stomatal entry by germ tubes of *B. lactucae*. It is all the more surprising therefore that, of the many penetrations by this fungus which I have observed, none has been through a stoma.

Young lettuce leaves were inoculated with a strong suspension of *Bremia* spores placed in Petri-dish moist chambers and incubated over the temperature range 1–25° C. After 24 hr., they were cleared in acetic acid : alcohol (1 : 3) for 3 hr., and left in alcohol : lactophenol (1 : 1) overnight. Next day they were stained in 1 % aniline blue in 95 % alcohol and examined microscopically.

Infection had taken place in 24 hr. at all the temperatures used except 25° C. Typical penetrations are shown in Figs. 1–3 which relate to 20° C. Cuticular penetration is all the more striking when it takes place close to a stoma as seen in Fig. 2.

CROSS-INOCULATION EXPERIMENTS

B. lactucae is known to occur on a number of composite genera, e.g. *Crepis*, *Hieracium*, *Lactuca*, *Senecio*, *Sonchus* and *Tragopogon*. Previous workers (Ogilvie, 1946; Wild, 1947) had attempted to infect these with *Bremia lactucae* from the cultivated lettuce but succeeded in infecting only *Lactuca serriola*, *L. virosa* and *L. saligna*. Ogilvie found *Bremia* occurring naturally on *Lactuca serriola*, which thus constitutes a source of danger in the neighbourhood of market gardens.

In the autumn of 1951 seeds from a number of wild composites were collected. They were as follows: *Cichorium intybus* L.; *Crepis capillaris* Wallr.; *Hypochoeris radicata* L.; *Lactuca muralis* Fres.; *L. serriola* L.; *Lapsana communis* L.; *Leontodon autumnalis* L.; *L. hispidus* L.; *Senecio jacobaea* L.; *S. squalidus* L.; *S. sylvaticus* L.; *S. viscosus* L.; *S. vulgaris* L.; *Sonchus asper* Hill; *S. arvensis* L.; *S. oleraceus* L.; *Taraxacum officinale* Weber; *Tragopogon pratensis* L. Seeds of these were grown in boxes to the two- or three-leaf stage, at which they were sprayed with a suspension of *Bremia* spores from cultivated lettuce. After the usual two wet periods of incubation, conidia appeared on the two *Lactuca* species listed and on cultivated lettuce (var. Trocadero Improved). The degree of infection was, however, very different, being 100 % on cultivated lettuce, 25 % on *L. serriola* and 5 % on *L. muralis*. None of the other composite species developed downy mildew. A repetition of the experiment gave infection of the cultivated lettuce and *L. serriola* but not of *L. muralis*. Infections were also successfully transferred from the artificially infected *L. serriola* back to cultivated lettuce. It was not possible, because of scarcity of spores, to transfer infection from *L. muralis*.

In October 1951 *Bremia* was found occurring naturally on *Senecio vulgaris*. A suspension of spores from this source was inoculated on to seedlings of the species listed above and on to cultivated lettuce. Ten days later seedlings of all the *Senecio* species had developed mildew to the following extents: *S. viscosus* 100 %, *S. sylvaticus* 90 %, *S. squalidus* 40 %, *S. vulgaris* 20 %, and *S. jacobaea* 10 %. None of the other species developed mildew.

These results confirm those of previous workers as to the existence of biological races of *Bremia lactucae*. *Lactuca serriola* is a fairly common weed in the Thames Valley district, especially on waste ground around London,

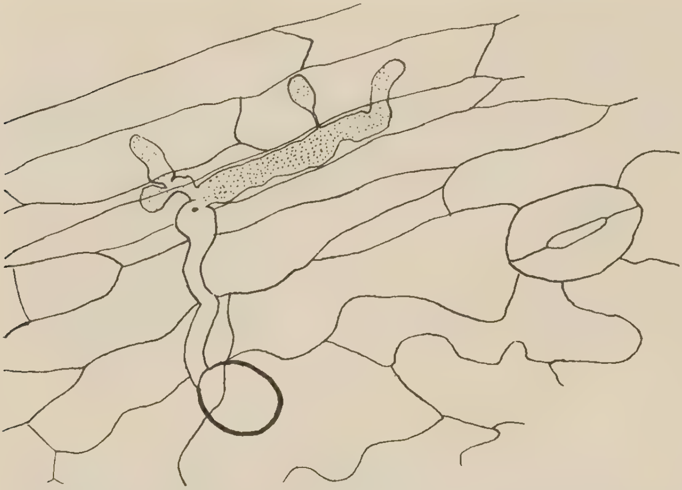

Fig. 1. After 24 hr. at 20° C.; internal mycelium well developed.

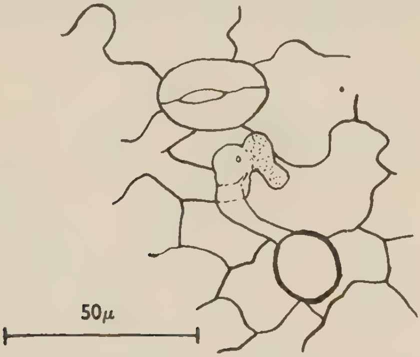

Fig. 2. As in Fig. 1; cuticular penetration close by a stoma.

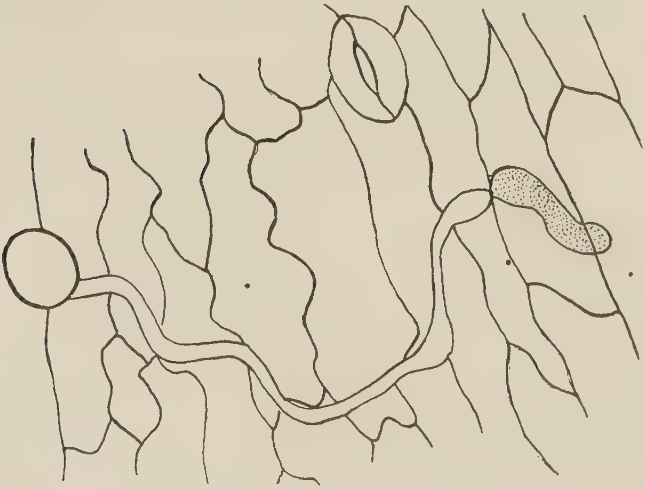

Fig. 3. As in Fig. 1; long external germ tube.

Figs. 1–3. Penetration of epidermis of lettuce leaf by germinating spores of *Bremia lactucae*.

and, being a biennial plant with a rosette of green leaves lasting over the winter, would be able to carry the disease over from one year to the next. It seems likely, however, that late summer lettuce and winter lettuce crops growing out of doors are more important in this connexion, as mildew lesions can often be found on such plants.

The author wishes to thank Prof. W. Brown, F.R.S., for suggesting the subject of this research and for assistance at various stages of the work; also the Department of Scientific and Industrial Research for a grant which enabled the work to be carried out.

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MICROSPORUM DISTORTUM SP.NOV. FROM NEW ZEALAND

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(With Plate 12)

A new species of *Microsporum* has been isolated from lesions of tinea capitis and corporis in the Otago district of New Zealand. The organism is distinguished by the distorted shape of the thick-walled, roughened macroconidia. It was possible to produce experimental lesions with it in laboratory animals and a human volunteer.

During routine investigations of the dermatophytes of the Otago district of New Zealand extending over the past seven years (1947 to 1953) we have isolated twelve strains of a hitherto undescribed species of *Microsporum*, which we propose to name *M. distortum* because of the distorted shape of its macroconidia.

Microsporum distortum sp.nov. (Plate 12)

Culturae in agaro Sabouraudi crescunt. Culturae aliae albae et lanosae, aliae cereae; subter flavae sunt. Microconidia piriformia in latere feruntur, $5-10 \times 2-3\mu$. Macroconidia aspera, densis parietibus, multiseptata, distorta, $30-60 \times 12-27\mu$. E tinea capitis liberorum, e tinea corporis liberorum adultorumque in Otagone, Novae Zelandiae.

Infected hairs covered with a mosaic of small spores and showing a green fluorescence under Wood's light. Gross appearance of cultures on Sabouraud agar variable; often white, raised and fluffy, with a tendency to become crateriform with age, but sometimes the aerial mycelium is scanty or lacking, so that the growth has a waxy appearance. A dull yellow pigment on reverse. Both macro- and microconidia present in cultures. Microconidia pear-shaped, borne laterally, $5-10 \times 2-3\mu$. Macroconidia thick-walled, rough, multiseptate, distorted, $30-60 \times 12-27\mu$. Favic chandeliers may occur. From tinea capitis of children and tinea corporis of children and adults in Otago, New Zealand.

The type (a strain isolated from a case of tinea capitis early in 1953), comprising infected hairs, slide cultures and a herbarium preparation, has been deposited at the Commonwealth Mycological Institute, Kew (Herb. I.M.I. 55048).

Nine of the twelve strains so far isolated came from scalp ringworms of children under the age of puberty, two from skin lesions of children, and one from the skin lesion of an adult. Ten of the infected individuals lived in country districts or small towns, and one in the urban area of Dunedin. The home of the twelfth was not known. The lesions caused by the organism are not clinically distinguishable from those due to *M. canis*, which in this region is responsible for almost all of the fungus infections of the scalp and hairy skin (Marples, 1951). In only one of the cases of tinea capitis was there any degree of inflammation, the child having a boggy, spreading lesion of 1 month's duration when referred to us. In the remainder of the scalp infections the principal symptom was the breaking off of infected hairs a short distance above the roots, accompanied by a moderate degree of scaling and reddening of the peripheral skin. We were able to follow the course of two of the scalp ringworms from shortly after onset until final clearing of the lesions. With local treatment they cleared in approximately 4 months, a period of time similar to the duration of like infections with *M. canis*. In one of the cases of skin infection the lesion was raised, red and tense when seen a day or so after its onset. The lesions of the second case were flat, dry and scaly. No information was collected upon the appearance of the lesions of the third patient.

ANIMAL INOCULATIONS

Mice, rats, guinea-pigs, rabbits, a cat and a human volunteer were inoculated with cultures and/or infected hairs. The animals used were half grown males. The areas to be inoculated were first clipped of hair and then lightly scratched with a sharp needle to produce breaks in the skin without gross damage. The culture or hair was then rubbed lightly over the area. All animals inoculated with hairs developed lesions. Results from inoculations with cultures were rather poor. Table 1 summarizes the results.

Table 1. *Animal inoculation with* Microsporum distortum

	Inoculated with cultures		Inoculated with infected hairs	
	No. inoculated	No. developing lesions	No. inoculated	No. developing lesions
Mice	2	0	—	—
Rats*	2	0	2	2
Guinea pigs*	2	1	1	1
Rabbits*	2	1	1	1
Cat	—	—	1	1
Human	1	1	—	—

* The rats, guinea-pig and rabbit which were inoculated with infected hairs were those animals which had been resistant to infection with culture material.

DISCUSSION

We have placed this species in the genus *Microsporum* because of the greenish fluorescence of infected hairs when placed under a Wood's light, the arrangement of small spores in a mosaic outside the hair and, in culture, the presence of thick-walled, roughened, although distorted, macroconidia (Pl. 12, figs. 1, 2). The presence of favic chandeliers, best demonstrated in slide cultures (Riddell, 1950), was at first confusing, for Conant, Smith, Martin, Baker and Callaway (1944, p. 253) state that these 'are supposed to be of some diagnostic importance' in the identification of *Trichophyton schoenleini*. However, we have also found favic chandeliers in a dysgonic variant of *Microsporum canis*, where they formed almost all of the scanty aerial mycelium of the primary culture. Emmons (1934) states that the thick-walled, roughened macroconidium is typical of *Microsporum*, and that the macroconidia of *Trichophyton* species are thin-walled and smooth.

The distribution of this organism, apparently restricted to the province of Otago, is interesting. It does not appear to occur in and about the city of Christchurch in the South Island of this country, nor in the provinces of Taranaki, Hawkes Bay and Auckland in the North Island, for it has not been amongst the cultures of unusual pathogenic fungi which are sent to us for identification from laboratories in these areas. The first eight cases showed discontinuous distribution in both time and space. Prior to August 1953, fresh isolates of the organism appeared at fairly regular intervals, with no evidence of contact between patients. One child of pre-school age, who had a scalp infection, and who lived in the country, was said to have no contact with children other than his siblings who had no demonstrable lesions.

However, during August and September 1953 the organism was isolated from four cases of tinea capitis in children in a rather thinly settled farming district of Central Otago. Two of the cases were siblings who had no contact with the third case, nor with any children known to have lesions. The contacts of the fourth case were not known.

It may be possible to explain the apparently localized distribution of *M. distortum* by supposing that the species is of local and recent origin which has become established amongst the animals of the area. It seems likely that the animals involved are farm stock or feral rather than domestic pets, for nearly all the infections have been in country districts from which we see few patients, and only one was known to have originated in the Dunedin city area from which most of our patients are drawn.

We are indebted to Dr G. C. Ainsworth of University College, Exeter, for help in the preparation of this paper, and to Prof. G. R. Manton, Department of Classics, University of Otago, who prepared the Latin diagnosis.

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EXPLANATION OF PLATE 12

Fig. 1. Typical macro- and microconidia of *M. distortum*. Slide culture, × 660.

Fig. 2. Atypical macroconidia of *M. distortum*. Slide culture, × 660.

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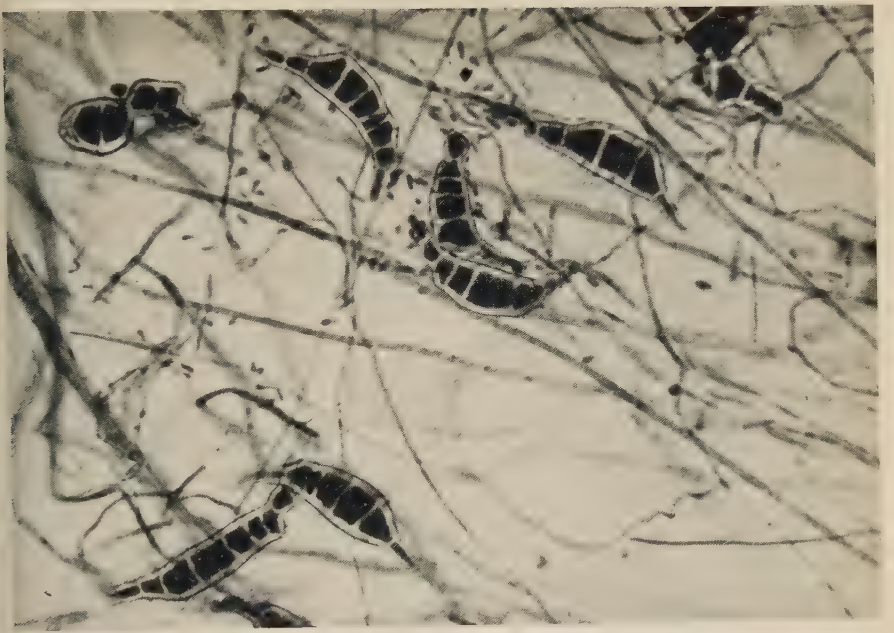

Fig. 1

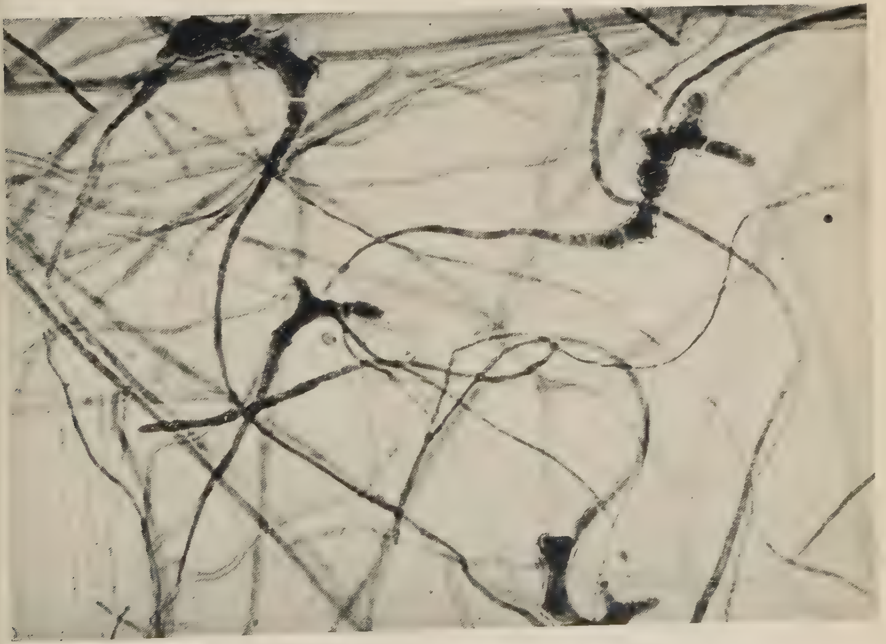

Fig. 2

PIPTOCEPHALIS XENOPHILA SP.NOV. PARASITIC ON NON-MUCORINE HOSTS*

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AND MARY P. ENGLISH†
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(With Plate 13 and 3 Text-figures)

A new species of *Piptocephalis* first found near Bristol in 1941, and again in Canada in 1951, described and figured in detail, is named *P. xenophila*. On both occasions it was parasitic on a species of *Penicillium* obtained from soil, and it has also been cultured on a number of other ascomycete moulds, on which it appears to grow better than on the Mucorales. This is so far the only mucorine parasite known to be able to form haustoria in a host of another Order. The new species is morphologically very distinct from all those previously described, especially in its minute head-cell and long spore-chains. The genus *Piptocephalis* has had scant attention since the turn of the century, and an attempt is made to clarify the nomenclature of the parts.

Piptocephalis xenophila sp.nov.

Mycelium tenue, ramos laterales multos exhibens eosque clavis simillimos. Sporophori erecti, circa 2 mm. alti, primo sine colore, deinde albi vel lutei. Caulis principalis nec striationes habet nec rhizoides nec stolones, plerumque non ramosus nisi quod prope apicem exstant rami densi et sporas ferentes. Cella capitalis minima, 2–3μ lata, aut parvo aut nullo latior quam qui eam fert ramus, cordis formam praebens ex latere visa, 4 aut 5 lobis distalibus conjunctis, quorum suam quisque sporarum catenam habet circa, 6 (nonnunquam ex 3–12) sporis compositam. Sporae, in aqua, priusquam turgent, cylindratae, 3·0–5·5$\mu \times$ 2·0–2·5μ, acervatae pallidae vel luteae. Parasitica in *Penicillio waksmani* ex terra arenosa crescens prope Bristol, in Anglia, Junio 1941. Potest autem ex ascomycetis et mucoraceis multorum generum parasiti modo crescere.

Mycelium fine with many peg-like lateral branches. Sporophores erect, about 2 mm. high, at first colourless, later cream or buff; the main stalk without striations, rhizoids or runners, generally unbranched except near the top, where it bears a compact system of sporing branches. Head-cell minute, 2–3μ wide, little or no broader than the branch bearing it, somewhat heart-shaped in side view, with 4 or 5 distal lobes each carrying a spore-chain of about 6 (range 3–12) spores. Spores (in water, before swelling) cylindrical, 3·0–5·5 by 2·0–2·5μ (average 4 by 2μ) pale buff in the mass. Parasitic upon *Penicillium waksmani* from sandy soil near Bristol, June 1941. Capable of parasitizing in culture a wide range of ascomycete and mucorine moulds.

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* This paper incorporates material from a thesis by M. P. English approved in 1943 for the M.Sc. degree of the University of London.

† Formerly of King's College, London.

The type specimen (Herb. I.M.I. 56692) and cultures have been deposited at the Commonwealth Mycological Institute, Kew.

The fungus here described was first seen growing in 1941 in a Petri dish of malt agar into which sandy soil from a site near Bristol had been blown. Various other moulds, including a *Penicillium* sp. and *Mucor* spp., were present, but a second 'blowing' (see Dobbs, 1942) separated off pure colonies of the *Penicillium* infected with the *Piptocephalis* which, in about a week, formed conspicuous cream-coloured sporophores rising above the host colonies (Pl. 13, figs. 1, 2). In October 1951 a second culture of what is undoubtedly the same species was received from Miss D. Wells, of the Canadian Department of Agriculture, Division of Plant Pathology, Ottawa. This also had been obtained from soil and was parasitic on a *Penicillium* sp. Dr Kenneth Raper, who has been good enough to examine the two hosts, considers that the first is best assigned to *P. waksmani* Zaleski and the second to *P. frequentans* Westling.

On 26 June 1943 we read a joint paper at the British Mycological Society's meeting at Bristol, concluding that the parasite is a new species of *Piptocephalis* and that it is unique in its range of parasitism. Both of us then had to drop work on the subject for some years, and it has been difficult to complete it. The reappearance of the mould, after ten years, in Canada seems to show that it is not merely a mycological curiosity and that it is probably widely distributed in the soil.

THE GENUS *PIPTOCEPHALIS*

The genus *Piptocephalis* was established by de Bary (1865) with the type species *P. freseniana*. Very little original work on it has been published since the turn of the century, and the nomenclature of the sporing parts is confusing, so that a brief résumé of what is known about it is necessary. Brefeld (1872) made *P. freseniana* the subject of one of his careful studies. van Tieghem & Le Monnier (1873) and van Tieghem (1875) described several new species. Bainier (1883), Vuillemin (1887, 1902), Mangin (1899) and Matruchot (1900) added to the list. After this period there was a long gap before another new species was described by van Beyma (1944).

Among the monographers of the Mucorales, Fischer (1892) includes eight species of *Piptocephalis*, basing his main subdivision of them on the presence or absence of 'stolons' and rhizoids; Lendner (1908) briefly lists nine out of the twelve species then published; Zycha (1935) bases his treatment on that of Fischer, pending further investigations, but reduces the number to seven by identifying some of those later described with earlier species, notably *P. monospora* Mangin and *P. tieghemiana* Matruchot with *P. cylindrospora* Bainier. Naumov (1939) retains twelve species, which he distinguishes mainly by characters of the sporing head and its parts.

The fungi of this genus are all obligate parasites on other moulds which they attack by means of haustoria growing usually from an external swelling on the fine mycelium. The relatively large sporophores may arise directly from this mycelium, but in three of the species accepted by Zycha they arise from 'stolons' supported by rhizoids. The sporophore may be smooth or striated, and is usually branched only near the top, where it supports an inflorescence-like series of regularly dichotomous branches, the ultimate tips of which each carry, when mature, a relatively short, broad cell (the 'head-cell'). This in turn bears a number of straight hyphal rods ('sporing-rods') which become septate and give rise to fragile 'spore-chains'. As the generic name indicates, the whole 'head' with its 'head-cell' is capable of dropping off.

Nomenclature of the sporing parts

Several workers have investigated the sporophore. Brefeld thought that the spores arise by simple septation of the sporing-rods; van Tieghem & Le Monnier (1873) that the spores are formed inside a sporangial wall which frees them by dissolution; Mangin (1899), using chemical reagents, found that each conidium has two distinct walls, so that the breaking up of the spore-chains is considered to be the separation of one-spored sporangia. Gäumann (1926) suggested that the 'head-cell' is derived from a much reduced sporangium of the *Mucor* type, the 'sporing-rods' being 'extra-sporangial partial sporangia', the walls of which are fused with the spore walls when the spores are cut off.

This variety in interpretation has led to chaos in the naming of the parts. The cell which we have decided to call the 'head-cell' has been variously known as 'la tête' (Bainier, van Tieghem), 'der Knopfchen' (Brefeld), the 'basidial cell' (Fischer), the 'basal cell' (Lendner, Zycha) and the 'sterigma' (Naumov). The 'sporing-rods' have been known as 'die Schäuche' (Brefeld), 'les bâtonnets' (Mangin and Lendner), 'les baguettes' (van Tieghem), 'sporangia' (Matruchot, Mangin, van Tieghem), 'Tielsporangien' (Gaumann, Zycha). Having an objection to the use of terms with implications which are necessarily speculative, we propose to use names for these parts which are, as far as possible, purely descriptive. The 'head-cell' is so called because it is the core of the 'falling head' which is characteristic of the genus. 'Sporing-rod' is the name used for the rod-like outgrowth before septation; 'spore-chain' for the same organ after septation.

Range of parasitism

Except for the new species here described, members of the genus *Piptocephalis* have not hitherto been found or cultured on any but mucorine hosts, and in fact this seems to apply to all the terrestrial moulds hitherto described which establish haustoria in the hyphae of other fungi. So far as we can discover all known fungal parasites of this type are Mucorales, and with this sole exception are restricted to Mucorales as hosts.

Matruchot (1903) made a study of the reactions of his species *P. tieghemiana* (*P. cylindrospora* Bainier, according to Zycha) with a wide range of other fungi as potential hosts, including one myxomycete, one oomycete, twenty-five ascomycetes (among which were species of *Penicillium* and *Aspergillus*), nine basidiomycetes, and a member of the Entomophthoraceae, on all of which the *Piptocephalis* failed to grow. He grew it successfully on ten members of the Mucoraceae and one of the Pilobolaceae, and came to the conclusion: 'en dehors des Mucorinées aucun champignon ne peut servir d'hôte à *P. tieghemiana*'. Consequently, he regarded the ability of the mould *Oedocephalum echinulatum*, described by Thaxter (1891) as a hyphomycete, to act as a host as evidence of its membership of the Mucorales, and established the genus *Cunninghamella* for its inclusion. Blakeslee (1905) later discovered the zygospores of *C. echinulata.*

Matruchot's conclusion was justified for the species which he used, but the behaviour of the new species will make necessary a modification of the general assumption about the genus, and about mucorine parasites as a whole, that they are necessarily restricted to hosts of the same Order.

AIMS AND METHODS

A detailed study of the *Piptocephalis* growing on *Penicillium* spp. was undertaken to establish the fact of its parasitism by demonstrating the haustoria, and to identify or distinguish the species, which has been figured and photographed in some detail because no recent detailed studies in the genus have been made. Further work was designed to establish the range of fungi which were capable of acting as hosts to this unique parasite.

The hosts were cultured at 18–20° C. on 2 % malt agar or other common media, and most of the microscope work was carried out on living material in slide cultures, usually made by the 'split-agar drop' method (Vernon, 1931). For some observations on the haustoria the mycelium growing on the cover-slip was fixed in Navaschin's fluid and stained in Delafield's haematoxylin, and some mounts in cotton blue and lactophenol were used for observations on the sporophore.

Ordinary inoculation invariably transferred both host and parasite, but we successfully separated the parasite from one host for transference to another by several methods; either by lightly touching a dense growth of sporophores standing well above the host with a platinum wire or with a sterile cover-slip, and incubating the transfers, discarding those which showed infection by the first host before inoculation with the second; or by blowing the *Piptocephalis* conidia off a spore-retaining host on to a growing colony of a recognizably different mould.

Attempts to obtain single-spore isolations of the *Piptocephalis* were unsuccessful, but the blowing method gave a scatter of groups of conidia obviously derived from single 'heads', and in many cases from single spore-chains (Pl. 13, fig. 1) so that isolation from such colonies gave a reasonable certainty of strain purity. A separation of single conidia was obtained by a blowing of the *Piptocephalis* from a spore-retaining host to small drops of agar on a slide, but the transfer of these infected drops to colonies of a suitable host has not, so far, resulted in the establishment of the parasite.

The morphology of the *Piptocephalis* was studied mainly on the original host (*Penicillium waksmani* Zaleski), on *P. roqueforti* Thom, which, owing to its low growth, was a suitable host for transfers by the contact method, on *Aspergillus* sp. (*A. repens* series) and on *Mucor hiemalis* Hagem. Many other fungi were used in the investigation of its range of parasitism.

MORPHOLOGY AND LIFE HISTORY

On a suitable *Penicillium* host the sporophores of *Piptocephalis* appear 4–7 days after inoculation and spread over the host colony, eventually obscuring it though never spreading beyond its limits. On mucorine hosts a few sporophores appear after 5 or 6 days, but afterwards growth is slow and the host seldom becomes thickly covered.

The mycelium is very fine, of an average width of 2–3 μ, forming a network among the coarser branches of the host (Pl. 13, fig. 5). Its appearance is characterized by short peg-like lateral branches (Text-fig. 2; Pl. 13, fig. 4). No zygospores have been found, and so far as can be seen the two strains collected from nature do not react sexually together.

The spores and their germination

The spores of the new species are cylindrical with smooth walls, pale buff in the mass, of average size 4 × 2 μ (range of length 3–5·5 μ) (Pl. 13, fig. 6).

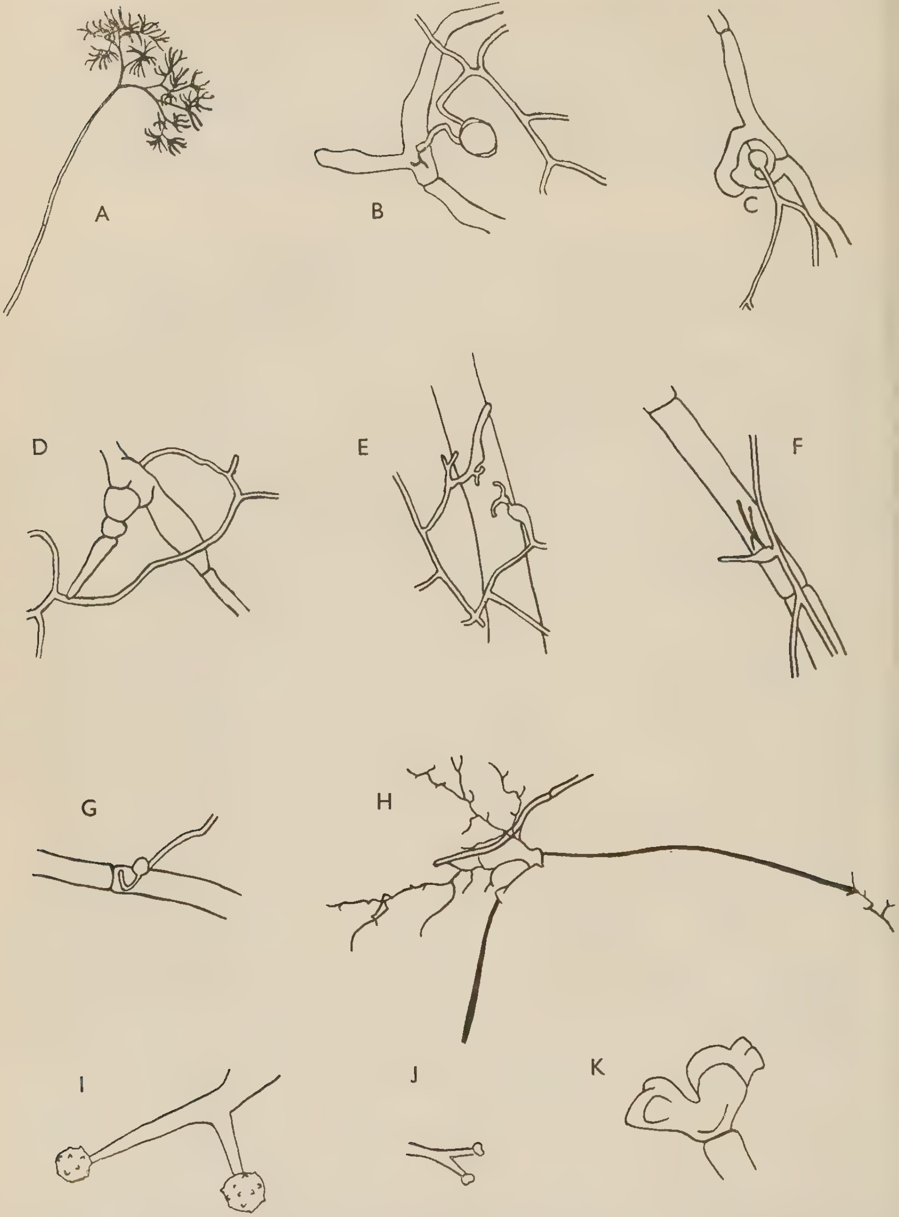
On the first day after inoculation the *Penicillium* spores swell and germinate, those of the *Piptocephalis* swell without germination, becoming roughly spherical, but showing temporarily at each pole a cap of a substance which appears to be less ready than the rest of the wall to absorb water (Text-fig. 1 B; Pl. 13, fig. 7). That these caps represent the points of disjunction of the spore-chain was established by observing the swelling of an unseparated chain of spores. This observation confirms that of Brefeld (1872) who figured such caps on swollen spores of *P. freseniana*. In our species the caps may remain visible on swollen spores for 2 or 3 days, although the time from the initiation of the sporing-rod to the breaking up of the spore-chain may be only one day. It seems likely, therefore, that the septa laid down between the spores are of a slightly different material from the wall of the sporing-rod, and that it is not merely a question of the longer exposure of the rest of the spore-wall to the external moisture. Germination may take place while the caps are still visible, or after they have stretched and merged into the rest of the wall.

Germination begins on the second day. Each spore may put out from one to six germ tubes which may grow in any direction, not necessarily towards the nearest host hypha (Text-fig. 1 B; Pl. 13, fig. 8) which may be some distance away; but unlike the spores of *P. freseniana*, as described by Brefeld, those of our species will not germinate in the absence of the host.

Penicillium hyphae sometimes appear to be irritated by ungerminated *Piptocephalis* spores lying against them. They put out lateral branches which coil round the spores (Text-fig. 1 C) and may form a tangled mass of mycelium about them or may form obvious swellings (Text-fig. 1 D; Pl. 13, fig. 5). Such galls are often present in one part of a *Penicillium* hypha, while another part of the same hypha, apparently under exactly similar conditions, is not stimulated. Mature hyphae of *Penicillium*, sometimes bearing conidiophores, have been seen also to twine round the *Piptocephalis* sporophores (Text-fig. 3 I). Similar association reactions between *Penicillium* spp. and the parasitic Mucorales have been mentioned by Weindling (1938).

Germ tubes and mycelium

The germ tubes grow until they reach their host. If this is only a short distance away they grow straight without branching but if further they send out short lateral branches which give the characteristic appearance



Text-fig. 1. *Piptocephalis xenophila.* A, habit of mature sporophore (× 123). B, germinated spore showing the 'caps' and a germ tube with terminal haustorium. Host, *Penicillium waksmani* (× 893). C, gall formed by *Penicillium roqueforti* in contact with a *Piptocephalis* spore (× 440). D, gall formed by *P. waksmani* in contact with *Piptocephalis* mycelium (× 640). E, appressoria with two-branched haustoria on a hypha of *Mucor hiemalis*; stained (× 893). F, appressorium with three-branched haustorium on *P. roqueforti*; stained (× 893). G, unbranched haustorium on *P. roqueforti*; fresh (× 893). H, stolon-like structure in a culture of *Piptocephalis* on *Aspergillus repens* (× 90). I, J, K, head-cells of *Piptocephalis cylindrospora*, *P. xenophila*, *P. freseniana*, respectively; from fresh material (× 833).

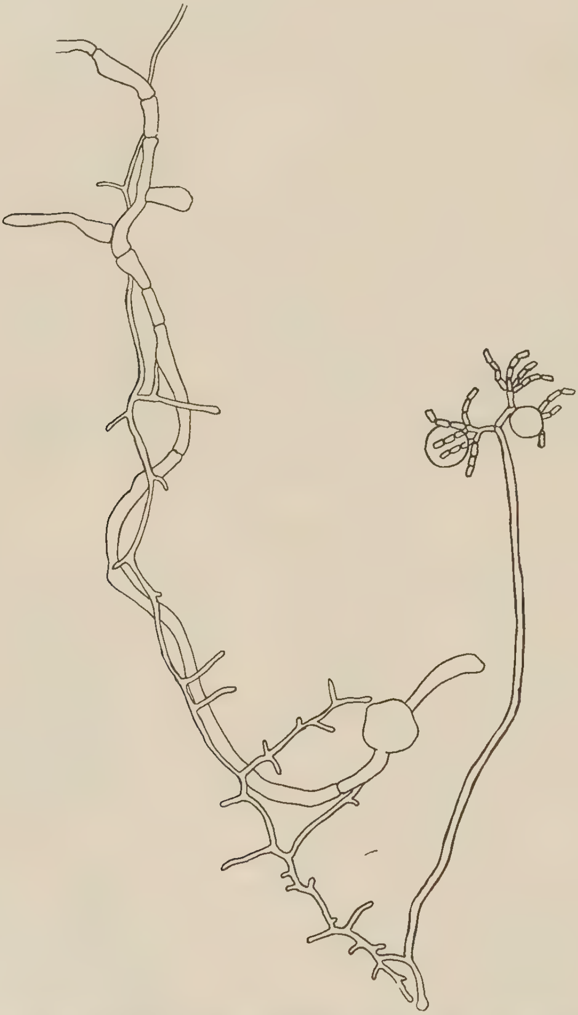
to the mycelium (Text-fig. 2). On reaching the host the germ tube may stop growth while haustoria are being formed, and this stoppage may be permanent, other germ tubes forming the main mycelium; or growth may be resumed, the hypha attacking other parts of the host in the same way. Frequently the hypha will turn aside and grow parallel to one of the host hyphae, sending out lateral branches in all directions (Text-fig. 2). Where these laterals touch the host haustoria are usually sent into it. In this way the parasite establishes a fine, close network of mycelium among the coarser branches of the host, which is not prevented from sporing however dense the growth of the *Piptocephalis*. Zycha (1935) remarks, in his description of the genus, that it attacks only the mycelium and not the sporophores of the hosts, and this is generally true also for the new species, but we observed one case in which haustoria were formed in the stalk of a conidiophore of an *Aspergillus* (*A. repens* series).

Swellings are often formed on the hyphae of a *Penicillium* host, at or near the point of attack, and there may be slight deformations at intervals along the whole length of a host hypha (Text-fig. 2). These galls are unlike those formed when a *Piptocephalis* spore lies near a hypha of *Penicillium*, for no new branches are put out, and there is no coiling round the parasite. They have not been seen when *Mucor* is the host, and they do not resemble the galls formed by *P. freseniana* on *Mucor*, as described by Brefeld, in which the host puts out a clump of short branches.

Appressoria and haustoria

The haustoria which the parasite puts into the host arise usually from an external swelling, which varies very greatly in size, applied closely to the host hypha. These appressoria are generally largest when they are applied to coarse hyphae (Pl. 13, fig. 10) such as are found in mucorine hosts, and small or indistinguishable on narrow hyphae such as those of *Penicillium* spp. (Pl. 13, fig. 9). They usually terminate, or lie near the end of, short lateral branches put out by the main hypha running parallel to the host (Text-fig. 1 E), but occasionally the main hypha may itself send in haustoria directly, where it passes over a hypha of the host (Pl. 13, fig. 9) or it may end in an appressorium (Text-fig. 1 G). It seems probable that this wide variation in the size of the appressoria is an unusual feature, corresponding to the wide host range in the new species, for it has not been noticed in other species, and the drawings of *P. freseniana* (Brefeld, 1872) and of *P. microcephala*, *P. cruciata* and *P. sphaerospora* (van Tieghem, 1875) all show appressoria of about the same size, although this may have been due to the choice of easily seen structures for drawing.

A single tube from the appressorium penetrates the wall of the host hypha and branches inside to form the haustoria (Text-fig. 1 E and F; Pl. 13, figs. 11, 12). These last are fine threads, the coarsest being something under 1 μ wide, although the lens effect of the host hypha sometimes makes them appear broader than this. The size of the haustoria seems to bear no direct relation to the size of the host hypha or of the appressorium. The number of haustorial threads arising from one appressorium is also variable, there being sometimes only one curved branch, sometimes two and sometimes three or four (Text-fig. 1 G, E, F). Branching may take place either at the tip of the piercing tube or further along the length of the haustorium as figured by Brefeld for *P. freseniana*, but in their thicker and fewer branches the haustoria are more like those figured by van Tieghem (1875) for *Dispira cornuta.*



Text-fig. 2. *Piptocephalis xenophila.* Young mycelium bearing a small sporophore, and attacking a young hypha of *Penicillium roqueforti* which is slightly swollen at intervals (× 440).

Despite the comparatively large size of some, these haustoria were very difficult to see as they tend to be obscured by the hyphal contents, especially in *Penicillium* spp. (Pl. 13, fig. 9). Occasionally they are conspicuous in a hypha with clear contents, even in the living state, but in general, where the contents are not too dense, staining improves their definition (Pl. 13, fig. 12).

The sporophore

Detailed observations on the living sporophores were carried out in slide cultures with a $\frac{1}{6}$ in. objective. The restricted space made the sporophores grow horizontally, but so long as they did not touch the cover-slip their development was normal. Where a branch-tip or sporing-rod touched the cover-slip its development was arrested, but the growth of the rest of the sporophore remained unaffected.

The mature main stem of the sporophore has a smooth, faintly buff-coloured wall, and is not supported on 'stolons' or rhizoids. It is a relatively massive structure, typically but not always narrowed towards the base where it arises from the fine basal mycelium—often, as shown in Text-fig. 2, from the small side branch to a main hypha formed by the original germ tube.

When the fertile hypha has reached its full height it forks at the tip, and continues to branch dichotomously from two to six times, each 'dichotomy' being shorter and narrower than the last, and the final branches sometimes no more than 4μ in length (Text-fig. 3A). The sporing-rods then appear as four or five minute buds at the tip of each branch and continue to grow in length for about $1\frac{3}{4}$ hr. (Text-fig. 3B–E). About an hour after they have reached their full length drops of liquid are exuded among them for about a quarter of an hour. Very shortly after this septa appear simultaneously, faintly at first, across the rods, dividing them into spore lengths, and at about the same time a septum becomes visible across the tip of the fertile branch, cutting off the head-cell (Text-fig. 3 F–H, K–M; Pl. 13, fig. 14).

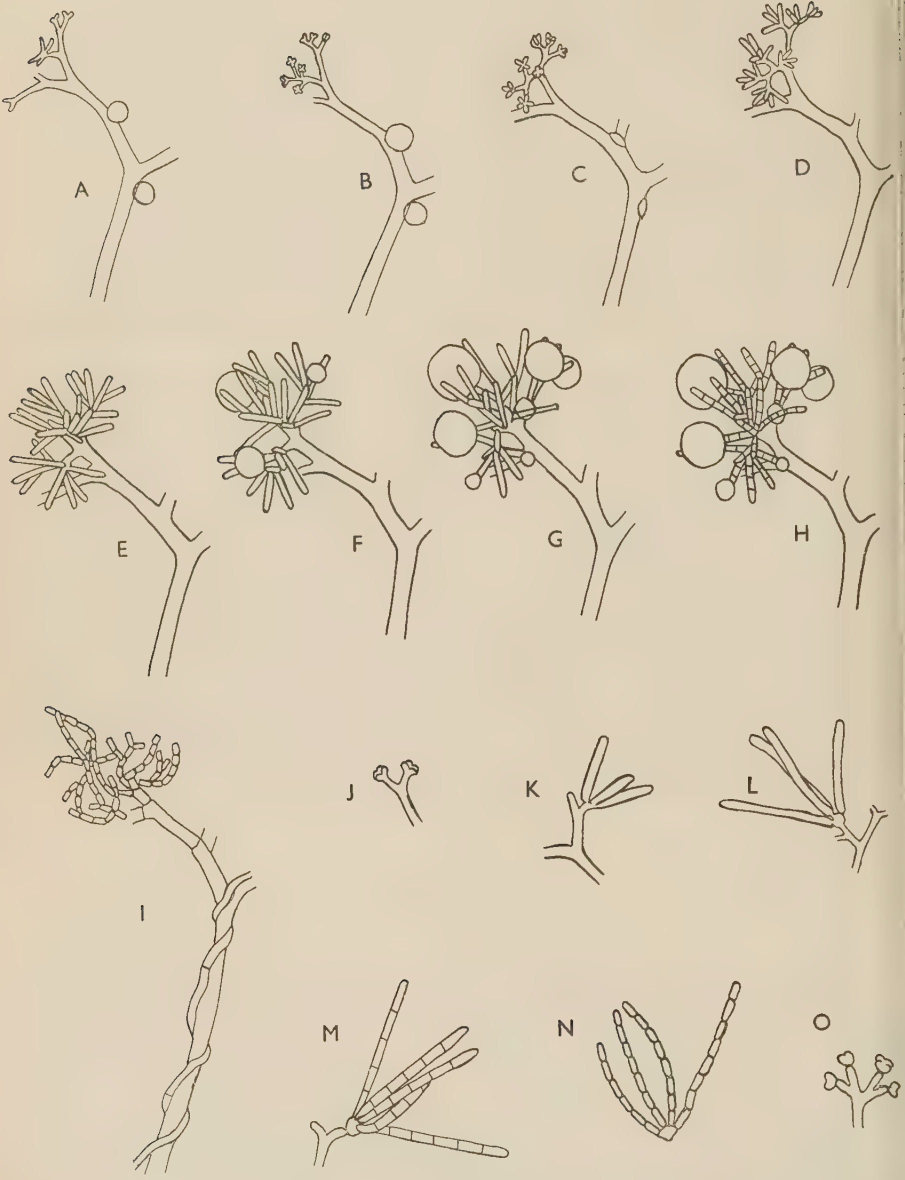
After the septation the spore walls gradually thicken and round off until the spores remain attached to each other only by a small portion of the middle of the septum (Text-fig. 3N). The average number of spores in a chain, from a count of 100 spore-chains, is six:

Number of spores in chain	3	4	5	6	7	8	9	10	11	12
Number of chains	0	5	25	37	20	8	3	1	1	0

Average 6·2±0·13, range 4–11.

Isolated observations on other occasions increased the range to 3–12.

As the spores round off, the drops of liquid exuded among the rods dry up. That these drops are, for the most part, exuded and not merely condensed on the sporophores, seems probable because they make their appearance always at the same stage in the development of the sporophore. The larger drops may envelope two or three spore-chains from the same or neighbouring heads, but almost invariably the spore-chains remain on the surface of the drop, so that, as it shrinks in drying out, the surface tension breaks up the chains, leaving the head as in Text-fig. 3I, presumably a preliminary stage to the distribution of the spores by air. Only rarely has a drop been seen to wet and enclose a whole head, forming a spore-drop, as in *P. freseniana* (Brefeld, 1872, Taf. V, fig. 9). The smaller drops often migrate to the ends of the spore-chains, where they dry up leaving the chains intact. In general, when a fresh plate culture is exposed to the air, the drops rapidly dry up, and broken chains of spores are left in their place.



Text-fig. 3. *Piptocephalis xenophila.* A–I, stages in the development of a living sporophore, showing the growth of the 'rods' and their septation, the appearance of drops and (I) the broken spore-chains after evaporation of the drops (× 440). Times in minutes from the previous drawing: B, 7; C, 18; D, 20; E, 60; F, 55; G, 15; H, 30; I, 12 hr. J–N, drawings from fixed material to illustrate the development of the head-cell (× 893). O, head-cells retained on part of a sporophore after the fall of the spores (× 893).

The head-cell is minute, only 2–3 μ across (Text-fig. 1 J; Pl. 13, figs. 14, 6) as compared with 6 μ for *P. cylindrospora* and up to 24 μ for *P. freseniana* (Text-fig. 1 I, K). It is roughly heart-shaped in side view, and has four or five lobes (Text-fig. 3 O), each of which bears a spore-chain. It is not usually cut off from the fertile hypha until the septa appear in the rods (Text-fig. 3 L, M), but it is possible, both in fresh material and in stained preparations, to see that the tip of the hypha has denser contents than the rest. When the spores are ripe the head-cell falls with them, and usually the head breaks up more or less completely, but occasionally it is found detached, and almost intact (Pl. 13, fig. 16).

Various theories have been entertained concerning the breaking up of the spore-chains in *Piptocephalis*. van Tieghem, and also Fitzpatrick (1930), refer to it as being due to the dissolution of the outer or 'sporangial' walls of the sporing-rods, van Tieghem & Le Monnier (1873) suggesting that the drops help to dissolve them. Matruchot's (1903) explanation that the 'sporangial' wall breaks at the point of juncture of the spores seems to accord better with our observations, though we cannot regard the presence of a distinct outer wall as conclusively proved.

The head-cell itself is very difficult to observe, as when it falls with the spores it is too small to identify with certainty when lying free on the slide. But if the sporophore is knocked just before the spores are ripe they will often fall, leaving the head-cell alone attached to its branch (Text-fig. 3 O). This sometimes happens when cultures are being handled for staining.

The mature sporophore eventually becomes septate at every dichotomy except the final one, and there are commonly three or four septa also in the main stem. The process usually starts at one or two forks at the same time as the septation of the rods, but is not completed until some time after the spores are formed, the order of appearance of the septa being quite irregular. The cross-walls are of the normal type, without the tubular perforations reported for *P. microcephala* (van Tieghem, 1875).

Various abnormal sporophores were noticed, especially on *Aspergillus niger* and *A. repens*. On this last host young sporophores, with a single fork at the tip, were seen to make contact with the medium, where each branch of the dichotomy reverted to normal mycelium (Text-fig. 1 H), thus giving an appearance similar to that of the 'stoloniferous' species. When the parasite was transferred back to a *Penicillium* host this abnormality disappeared.

PARASITISM AND RANGE OF HOSTS

Brefeld (1872) states that in *P. freseniana* germ tubes which soon cease growth are put out in the absence of a host. In the new species the majority of spores swell on a moist medium but have never been seen to germinate in the absence of another fungus. On twelve malt agar slide cultures carefully seeded with spores of the parasite alone, not one spore was found to have germinated after 6 days, although most of them subsequently germinated when the slides were inoculated with suitable host fungi. The same occurred (after 3 days) on maize extract agar and potato agar.

The need to incubate the *Piptocephalis* spores and to discard those slide on which the original host is present (which may be as many as half th total number) slows down the process of testing fungi as hosts, so that small number only could be tried. Table 1 divides them into five group according to the reaction of the *Piptocephalis* spores to them.

Table 1. *Host range of* Piptocephalis xenophila

Group I. *Fungi not attacked.* Piptocephalis *spores do not germinate**

- Penicillium claviforme Bain.
- Aspergillus sp.
- Botrytis sp.
- Chaetomium sp.
- Fomes annosus Fr.

Group II. *Fungi not attacked.* Piptocephalis *spores produce germ tubes or short hyphae*

- Achlya caroliniana Coker
- Brevilegnia sp.
- Saprolegnia sp.
- Pythium aphanidermatum (Edson) Fitz.
- Ceratostomella fimbriata Ell. & Habst.
- Ophiobolus graminis (Sacc.) Sacc.
- Collybia velutipes Quél.

Group III. *Fungi attacked.* Piptocephalis *forms a slow-growing mycelium, but sporophores are small and sparse*

- Cunninghamella echinulata Thaxt.
- Pilaira anomala (Cesati) Schröt.
- Mucor plasmaticus van Tiegh.
- Mortierella ramanniana (Möller) Linnemann

Group IV. *Fungi readily attacked.* Piptocephali *sporophores very sparse at first, though may become dense after some weeks*

- Mucor mucedo (Auct.) (+ and −)
- M. hiemalis Wehm. (+ and −)
- Absidia capillata van Tiegh.
- A. glauca Hagem (+ and −)
- A. spinosa Lendner
- Aspergillus niger van Tiegh.
- Macrosporium sarciniforme Cavara

Group V. *Fungi readily attacked. Dense masses o* Piptocephalis *sporophores appear in about a week*

- Byssochlamys fulva Olliver & Smith
- Cephalosporium sp.
- Diplodina sp. (from Delphinium ajacis)
- Aspergillus repens (Corda) de Bary
- Neurospora tetrasperma Dodge
- Penicillium waksmani Zal.
- P. frequentans Westl.
- P. camemberti Thom.
- P. notatum Westl.
- P. roqueforti Thom
- Venturia inaequalis (Cooke) Wint.

* In plate cultures, no growth of *Piptocephalis* was seen on *Conidiobolus villosus* Martin, *Pholiot adiposa* Fr., *Polystictus versicolor* Fr., and *Panaeolus* sp. No spore germination was observed, but i these cases the dense growth of the mycelium prevented effective microscopic examination.

Distinction between Groups I and II is often impracticable when the mycelium rapidly overgrows the spores. All that can be said in such cases is that an attack is not established. This can also be roughly tested by inoculating host and parasite together to a growing mycelium and observing whether or not the *Piptocephalis* spreads on the new host. If it appears to do so a more rigorous test is justified.

In Group III the sporophores produced in slide cultures were extremely small and sparse. On one culture with *Cunninghamella echinulata* there were two dwarf sporophores after 14 days, and no more 3 days later. When transferred to a tube the host soon swamped all signs of the parasite. On *Pilaira anomala* and *Mucor plasmaticus* the haustoria were exceptionally large.

In Groups IV and V the attack is vigorous, and may result in a dense growth of sporophores obscuring the host from view. The reason for the long delay in establishing this stage by the fungi in Group IV is unknown. On the two imperfect ascomycetes in this group the mycelium was denser than on the mucorine fungi, and the sporophores slightly more numerous.

The most significant fact shown in Table 1 is that all the fungi in Group V, those most strongly and rapidly attacked, are ascomycetes, whereas the Mucorales tested are all in Groups III and IV. On both occasions when, up to the present, the new species has been isolated from soil it has been found on a *Penicillium.* These facts indicate that this parasite is unique, not only in being able to attack non-mucorine hosts, but also in being more at home on ascomycetes, especially it seems on Aspergillaceae, than on members of the Mucorales.

No basidiomycete has been found to be attacked, and the germination of spores in the presence of *Collybia velutipes* is the sole example so far of any reaction with a member of that Class of the fungi. On the other hand, the mixed collection of phycomycetes and ascomycetes in Groups I and II indicates that, outside the Mucorales, immunity from attack may be largely independent of general genetic affinities. Even a *Penicillium* and an *Aspergillus* are completely immune. There are no Mucorales in Groups I and II, and the fungus shares with the other members of its genus the ability to attack a wide range of members of its own Order.

The assumption that *Piptocephalis* spp., and indeed mucorine parasites generally, are limited to the Mucorales as hosts is based partly on general report and observation, but especially on the work of Matruchot (1903) on *P. tieghemiana*, which appears to be the only previous published record of a systematic attempt to establish the range of hosts attacked by such a parasite. Matruchot does not describe his methods in detail, but it is evident that he used 'macroscopic' cultures only and he does not state how long he kept them under observation. Our first transfer of the new species of *Aspergillus niger*, for instance, produced no visible sporophores of the parasite for three months, and such a delayed attack might have been missed by Matruchot. At the same time, there is no evidence for even the feeblest attack on any non-mucorine host by any mucorine parasite other than the species here described.

Mucorine parasitism, with its restriction of host and parasite to the same natural Order, has hitherto been regarded as a special relationship, possibly quasi-sexual in nature, of limited interest except to students of the Mucorales; but this easily cultivated example of a phycomycete establishing haustorial contact with the protoplasm of a range of unrelated moulds may provide information which could conceivably throw light on the haustorial type of fungal parasitism generally, since the delays involved in the cultivation of the host can here be cut to a minimum, and the whole host-parasite system can be grown and observed on a microscope slide.

Systematic position

The only species previously described with a 'heart-shaped' head-cell is *P. microcephala* van Tieghem, 1875, but this is 'stoloniferous' and has perforated septa in the sporophore. The head-cells also are somewhat larger ($3-4\mu$) and the spore-chains shorter and adhere together when ripe. The spores are larger ($3 \times 6\mu$) and are described as 'dark gold' in colour. In August 1953 we received from the C.B.S. a culture of a *Piptocephalis* sp. sent there by Dr C. W. Hesseltine, which has head-cells and spore-chains similar to, but even smaller than, those of our species. This species, however, at present unnamed, is clearly distinguished from ours by its taller habit, 'stoloniferous' growth, and striated sporophores.

It is a characteristic of the minute 'heart-shaped' head-cell that it bears very few (usually 2–5) spore-chains. All members of the genus other than those mentioned above have relatively numerous spore-chains borne on a larger type of head-cell, either turbinate or globular in shape (Text-fig. 1 I–K). Cultures of several species available have been examined and it is evident that, quite apart from its unique range of parasitism, our species is morphologically distinct from those having either of these types of head-cell. Its salient characteristics are, the 'non-stoloniferous' growth, the minute 'heart-shaped' head-cell, and the few but long spore-chains. There is also a tendency for branching to be restricted to the upper part of the sporophore, which typically takes the form of a slender stalk bearing a relatively dense, symmetrical 'inflorescence' of creamy flower-like heads (Text-fig. 1 A; Pl. 13, figs. 2, 3). Its marked preference for certain hosts not in its own Order, or even Class, of the fungi, which is confirmed by its discovery a second time on a *Penicillium*, has suggested the epithet *xenophila*.

We are grateful for the Latin diagnosis to Mr A. O. Hulton of the University of Sheffield.

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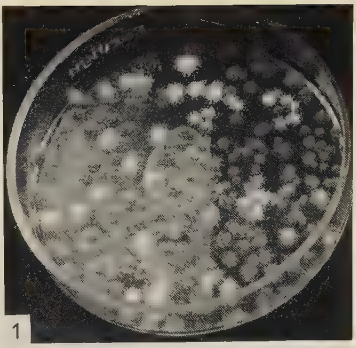
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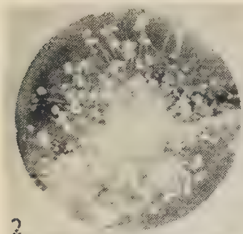
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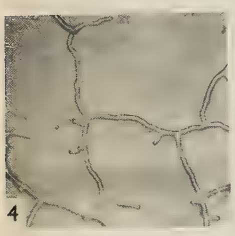
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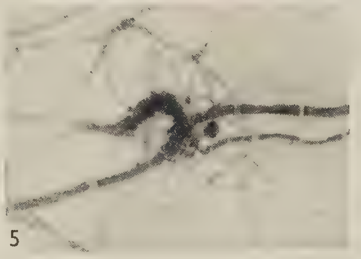
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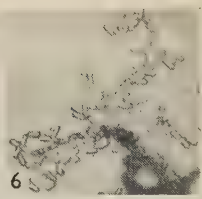
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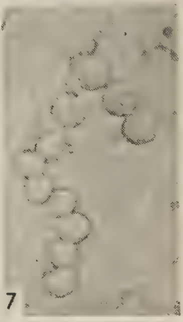
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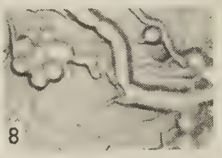
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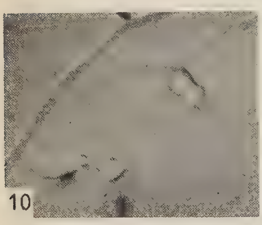
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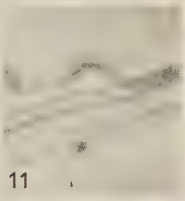
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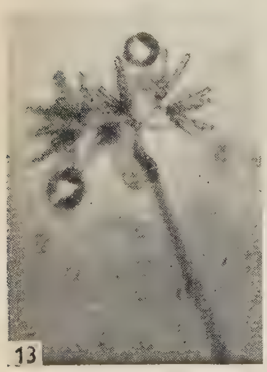
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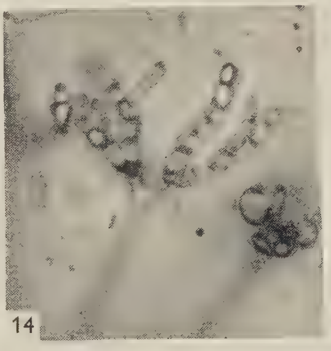
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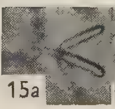
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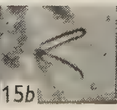
15*b*

15c

16

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EXPLANATION OF PLATE 13

Fig. 1. *Piptocephalis xenophila* growing on colonies of *Penicillium waksmani*; inoculated by blowing from an infected colony. × ½.

Fig. 2. Habit of sporophores. × 7.

Fig. 3. A single sporophore. × 70.

Fig. 4. Living mycelium of *Piptocephalis xenophila*. × 440.

Fig. 5. Stained mycelium of parasite and host (*P. waksmani*), the latter showing gall formation. × 80.

Fig. 6. Recently detached, living spores of the parasite, in water. × 440.

Fig. 7. *Piptocephalis* spores after swelling in water, with some, out of focus, still unswollen. × 880.

Fig. 8. *Piptocephalis* spores germinating near a hypha of *Penicillium waksmani*; living material. × 440.

Fig. 9. *Piptocephalis* hypha with faintly visible haustoria in a hypha of *Penicillium waksmani*, which has swollen slightly at the point penetrated; living material. × 1300.

Fig. 10. Large appressoria of *Piptocephalis* on a hypha of *Mucor hiemalis*; living material. × 880.

Fig. 11. Appressorium and penetration tube on *Penicillium roqueforti*; fixed and stained. × 1700.

Fig. 12. Long, fine, branched haustoria in *P. roqueforti*; stained. × 880.

Fig. 13. Sporophore of *Piptocephalis* showing unbroken spore-chains, and bearing drops. × 340.

Fig. 14. Part of a sporophore at a later stage, showing two head-cells each bearing four spore-chains; living material. × 1000.

Fig. 15*a*, *b*, *c*. Successive stages of spore-formation by septation of a sporing-rod; living material. × 660.

Fig. 16. A detached head which has lost some of its spores, but still shows the head-cell and two 8-spored chains; living material. × 1000.

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THE CONSTRUCTION AND USE OF A PORTABLE VOLUMETRIC SPORE TRAP

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(With 3 Text-figures)

For sampling air-borne spores a portable volumetric trap (which impacts spores down to 4μ diam. on a stationary glass slide for microscopic examination) is operated manually by a light sliding-vane pump. Samples of from 50 to 100 l. are taken in from 5 to 10 min. The apparatus can be used in places where a power supply, required for operating an automatic spore trap, is not available. Tests show it to be suitable for use in high spore concentrations, and in circumstances when an accurate time differentiation between samples is required. It is unsuitable for continuous operation.

CONSTRUCTION

The automatic volumetric spore trap, designed by Hirst (1952), can be operated continuously for the study of the composition and variation of the air spora. Two of its limitations are that it is not readily portable, and that it needs a source of power to drive the pump continuously. There is, therefore, a need for a portable, hand-operated, volumetric spore trap, which, while sacrificing many of the advantages of the Hirst trap, can be used where a power supply is not available.

The method of catching spores adopted for the portable trap is that used in the Hirst trap, which is based in turn upon the Cascade Impactor (May, 1945). The air to be sampled is drawn through a slit-shaped orifice-tube, and the spores are impacted on a sticky microscope slide. This arrangement, which has proved highly satisfactory in the Cascade Impactor and the Hirst trap, imposes limitations on the equipment that can be used for producing the requisite vacuum. The resistance at the orifice is too great for a light rotary blower, and the volume required is too great for a small piston pump. After tests with various kinds of pump and bellows it was found that a light sliding-vane pump† was satisfactory.

The portable trap (Fig. 1) consists of the following parts: (1) an impactor unit where, after passing through the slit orifice, the spores are deposited on a microscope slide; (2) a constriction, or orifice-plate, which stabilizes the rate of flow; (3) a low vacuum reservoir; (4) a vacuum gauge to measure the negative pressure in the reservoir; and (5) a manually operated sliding-vane pump to draw air through the system. These parts are connected by rubber tubing.

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† Mr J. C. Neill (personal communication) has successfully used a similar hand-operated pump in his studies of the rye-grass blind seed fungus in New Zealand. In other respects, however, his equipment was different.

The reservoir, vacuum gauge, pump and accessories are carried in a small haversack. The outfit weighs about 11 lb.

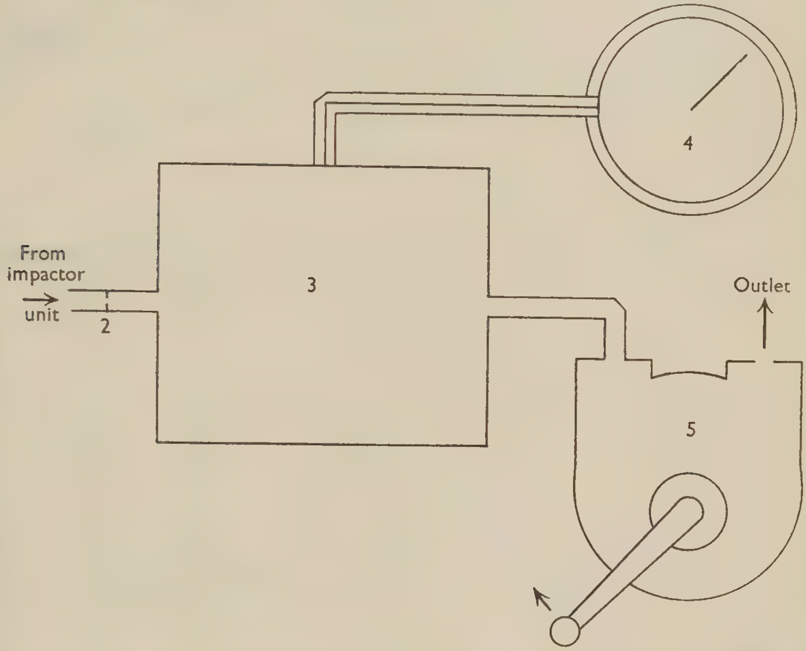

Fig. 1. Diagram of portable spore trap. Air drawn from impactor unit (Fig. 2) passes through orifice (2), to vacuum reservoir (3), and to sliding-vane pump (5) which is actuated by turning handle. The vacuum gauge (4) is connected to reservoir by a narrow capillary.

Orifice and impactor unit (Fig. 2)

The orifice of the impactor unit is a duct (*a*) 15–20 mm. long of rectangular cross-section 14 × 2 mm. It can most simply be made by beating a length of copper tube to fit over a brass 'form' which has been cut and filed to the required internal dimensions of the orifice. (It is best made, however, by milling out of brass, or by soldering brass 'angle'.) The leading edges, which face the wind, are bevelled on the outside.

The body of the impactor unit is a brass tube (*b*) 90 mm. long, 31 mm. (1¼ in.) external diameter, and thickness 12 s.w.g. (0·104 in.). The bottom of the tube is closed by soldering a brass disk (*c*) which carries eccentrically a short length of brass outlet tube (*d*) for the rubber connexion to the vacuum reservoir and pump. A slot is cut in the wall of the impactor tube at 40 mm. from the bottom, and the impactor orifice is soldered into this slot. Narrow brass strips (*e*) are screwed to the walls within the impactor tube, extending from the base plate to within about 10 mm. of the top, to act as guides for the microscope slide (*f*) on which the spores are impacted. The slide is pressed forward against these guides by a spring (*g*) screwed to the back of the impactor tube. The gap (*h*) separating the face of the slide and the back of the impactor orifice should be 0·6 mm. (Davies & Aylward, 1951).

For use the end of the sticky glass slide is pushed down to touch the base plate, and the open end of the impactor tube is closed by a rubber bung. (Alternatively, the open end can be closed by a stout, moulded rubber cap (*k*) of the type used for closing centrifuge tubes; the length of the impactor tube should then be reduced to 80 cm.) The eccentric outlet (*d*) on the base plate must be on the same side of the slide as the orifice. The impactor unit is connected to the reservoir and pump by about 4 ft. of rubber tubing.

Reservoir unit

With the pump connected direct to the impactor unit the rate of flow of air through the impactor orifice would be very irregular. To smooth the flow the pump is made to evacuate a reservoir which then draws air at a relatively uniform rate through an orifice of approximately 0·8 mm. diameter. This orifice-plate is made by drilling with a wide bit from both ends of a short length of aluminium rod until a thin wall separates the two cavities, and then putting a narrow central hole through this wall. The final adjustment of this hole is made by reaming out during calibration as explained below. The orifice-plate should be close to the pump and reservoir so that there is only a small negative pressure in the rubber connexion to the impactor unit. The reservoir should have a capacity of about 1 l. (a quart paint tin has proved convenient). The vacuum gauge, which is connected to the reservoir, has proved essential for obtaining reliable flow rates. A sensitive altimeter graduated at intervals of 20 ft. was found suitable and was obtained cheaply as aircraft surplus. A narrow glass capillary tube is inserted in the rubber tubing joining the altimeter to the reservoir in order to damp out pressure changes and prevent jerking of the indicator needle. Connexions are made with $\frac{1}{8}$ in. bore brass gas fittings and rubber tube.

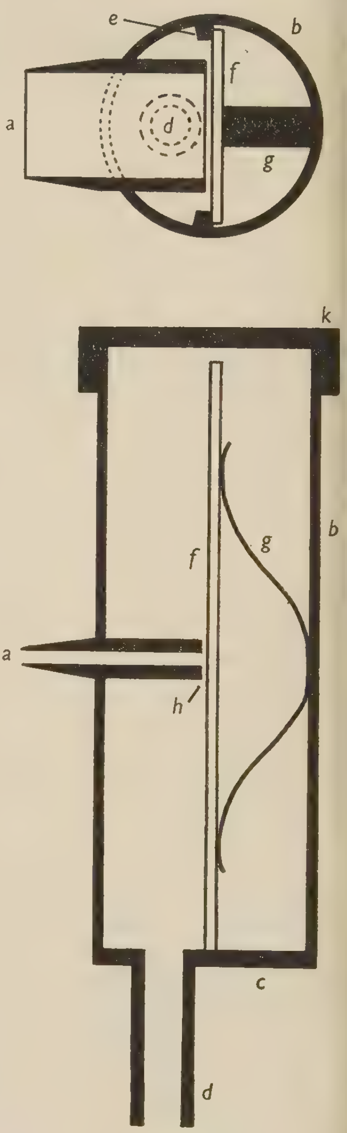

Fig. 2. Diagram of impactor unit in vertical and horizontal sections. (*a*) orifice duct; (*b*) body tube; (*c*) basal disk, carrying outlet tube (*d*); (*e*) guides for holding slide (not shown in vertical section); (*f*) glass microscope slide in position; (*g*) spring keeping slide in forward position against guides; (*h*) gap of 0·6 mm. separating back of orifice duct and front of slide.

Pump unit

The light sliding-vane pump can also be obtained cheaply as aircraft surplus. Connexion to the reservoir is made by rubber tubing to a brass tube in a rubber bung inserted into the suction port of the pump. The exit port is fitted with an oil trap and silencer. The pump is turned by a detachable handle, improvised from a retort stand boss, which can be rapidly screwed to the splined pump shaft. The other jaw of the boss carries a free-running handle made from a cut-down bicycle pedal crank.

Assembly

For field use it has proved convenient to pack the pump, reservoir and vacuum gauge tightly into a strong haversack lined front, bottom and back with sponge rubber. A piece of hardboard cut to fit one side of the haversack is screwed to the face of the pump. The splined pump shaft projects through both the board and side of the haversack. The handle is detached when not in use.

Calibration

The apparatus is calibrated by temporarily connecting the impactor orifice by soft, wide rubber tubing to a 'Rotameter'; then turning the pump handle steadily, and finding the vacuum gauge reading required to draw 10 l. of air per minute through the apparatus. With a plate orifice of approximately 0·8 mm. diameter in position, a negative pressure in the reservoir corresponding to an altimeter reading of +2000 ft. (=about 50 mm. Hg) gave a flow of 10 l./min. measured by a 'Rotameter' on the intake side of the impactor unit. The diameter of the small hole in the smoothing orifice can be adjusted to give a convenient reading on the vacuum gauge. From 60 to 70 turns of the handle per minute were required to obtain the pressure, depending on leaks in the pump and connexions. The volume of air at n.t.p. drawn through the orifice by each turn of the pump depends on the pressure in the reservoir and therefore on the rate of turning the handle. Counting the number of turns is therefore not an accurate measure of the volume sampled but it can be used as a makeshift in the dark, or if the speed of turning is timed by a watch. Regular calibration against a 'Rotameter' or other suitable flow meter is a necessary precaution against defects in the equipment.

The trap in use

The vanes of the pump work in oil, and about 10–20 ml. of liquid paraffin (B.P.) are added, as this is pleasanter to use than engine oil. Oil is lost fairly rapidly from the outlet port in the form of spray droplets, and the pump will need topping up after every 2 or 3 hr. running time.

When sampling, the impactor orifice is pointed towards the general direction of the wind. The impactor unit must be supported in the required position upwind of the operator; the type of support depends on circumstances. An aluminium spike 20 cm. long may be pushed into the ground and the impactor unit attached to it by a spring clip. For use at greater heights up to 4 ft. a single detached leg of a telescopic camera tripod can be slipped over the spike and the impactor unit clipped to the top.

The pump handle can be turned conveniently while the haversack is in the usual carrying position—no other support is needed.

With an impactor of the dimensions described here a sampling rate of 10 l./min. is probably the best compromise with the ideal sampling requirement, and this rate should be adhered to as closely as possible. The sample is timed by a watch to the nearest second. In practice a sample of 100 l. (10 min.) has been the maximum volume attempted. In most conditions 50 l. (5 min.) is adequate, and the pump handle can if necessary be worked for as many as sixteen to twenty 5 min. periods in 2 hr.

Preparing and examining slides

Slides are prepared for exposure as described by Hirst (1953), except that only the middle third of the length of the slide need be made sticky. The glass surface is first keyed with a thin layer of 10 % aqueous 'Solvar' (partially hydrolysed polyvinyl acetate) and allowed to dry. The slide is then warmed and a thickish layer of molten 'Vaseline' spread evenly over the prepared surface and allowed to cool; the edges are then scraped clean to make mounting easier.

Slides so prepared can be stored in a small slotted micro-slide box, but for field work in dusty places they are better carried in pairs back to back in aluminium tubes 1 in. in diameter, each closed with a rubber bung. Slides can be put into and removed from the impactor without special precautions.

After exposure the spore and dust deposit can usually be seen as a trace about 15 mm. long by 3 mm. wide across the middle of the slide. Exposed slides can be stored for months without deterioration just as removed from the impactor unit.

For microscopic examination it is necessary to mount the trace. Two or three drops of lactophenol 'Solvar' with a little cotton blue are placed on a thin, $\frac{7}{8}$ in. square cover-glass, and the slide lowered on to it. The trace can then be examined at once, but if possible it should be left to harden for a day or two before using an oil-immersion objective.

The density of deposit varies in different parts of the trace, and unless time is available to scan and count the whole trace, it is necessary to sample regions of different density. This can most conveniently be done by making ten or twenty traverses equally spaced across the long axis of the trace.

Examples of results

During 1953 the portable spore trap was tested to get information on its suitability in various applications. The examples selected below show the type of results that can be obtained and illustrate some of the difficulties encountered.

(1) *Simultaneous duplicate sampling with two similar traps*

6 August 1953, 11.45–12.23 B.S.T.; at 12 m. above ground-level in lattice tower in Meteorological Enclosure, Rothamsted Experimental Station, Harpenden. Wind west, Beaufort Force 3; sky overcast with occasional slight drizzle.

Two similar portable traps (P and L) were used with their impactor orifices placed 10 cm. apart facing the wind. A sampling rate of

10 ± 0·5 l./min. was registered by the gauge, but, to ensure that during the five consecutive duplicate runs the same volume was sampled by each of the traps, the pump handles were turned in time with a metronome beating 60/min.

This test was done to compare the readings from two similar traps with orifices close enough together to sample as nearly as possible from the same air. The samples were taken high enough above the ground for the distance of the nearest possible spore source to be 200 or 300 times the distance between the two orifices, so that variations in spore concentration could be smoothed by turbulence before reaching the trapping point. Each slide was scanned twice under a magnification of 720 diameters, making two sets of alternating traverses across the trace (counts A and B of Table 1). The spora consisted mainly of *Cladosporium* (mean: 6400 spores/m.^{3}, 3200 clumps/m.^{3}); and the total numbers of spores and clumps of *Cladosporium* counted on each set of traverses are shown in Table 1. Coloured basidiospores (1200/m.^{3}), spores of *Polythrincium trifolii* (260/m.^{3}), and spores of the *Botrytis* type were also present, but data for *Cladosporium* only are considered sufficient for comparison.

Table 1. *Two spore traps operated simultaneously in tower at* 40 *ft. Rothamsted Experimental Station,* 6 *August* 1953. *Orifices* 10 *cm. apart*

(Spore type: *Cladosporium* only. S = spores and C = clumps.)

	Trap P				Trap L					
	Count A		Count B		Count A		Count B		Totals	
Time G.M.T.	S	C	S	C	S	C	S	C	S	C
10.45	19	4	9	6	21	8	17	11	66	29
10.53	22	9	26	10	26	8	13	7	87	34
11.01	14	9	22	15	14	11	14	9	64	44
11.10	21	11	14	8	10	6	19	8	64	33
11.18	8	7	21	9	15	7	17	10	61	33
	84	40	92	48	86	40	80	45	342	173
								Mean	17·1	8·65

The data are interpreted as showing that two individual traps built of similar components give reasonably reproducible results when sampling the same spore cloud, and that when other errors, such as non-random distribution due to clumping, are eliminated, only random sampling errors remain.

(2) *Comparison with automatic volumetric spore trap*

The Hirst trap gives a deposit 12 times as dense as that obtained from a 5 min. sample with the portable trap. The deposit across any line of the Hirst trap represents the mean concentration during the hour, and its position can be determined with an accuracy of not less than ± 0·5 hr. (The accuracy of timing the arrival of any individual spore, however, can only be determined to ± 1 hr.) The automatic trap thus smooths the short period fluctuations which the 5 or 10 min. samples of the portable trap reveal (see §5). It is therefore of interest to compare the results obtained from short time samples of the portable trap with the smoothed data from hourly samples given by the Hirst trap. Several comparisons were made during the summer of 1953, while the two traps were used side by side on Great Field, Rothamsted Experimental Farm, Harpenden (Table 2).

Table 2. *Spores/m.³ at approx. 2 m. a.g.l.*

(P = concentration estimated by portable trap; H = concentration estimated by Hirst trap.)

Date ...	22 May		28 June		29 June		13 August	
Time (G.M.T.) ...	14.00		06.00		01.30		05.00	
	P*	H	P	H†	P	H	P	H
Pine pollen	260	410	—	—	—	—	—	—
Tree and grass pollen	635	420	—	—	—	—	—	—
Basidiospores:								
Coloured	75	210	100	420	8,250	1,000	500	40
Hyaline	25	50	9,750	6,500	3,000	1,410	500	460
Tilletiopsis type	—	—	8,250	1,100	0	700	0	80
Cladosporium	790	1,400	1,500	1,000	2,250	1,000	3,000	1,800
Fusiform ascospores	25	80	0	0	7,500	590	250	40
Total (including types not in above categories)	1,560	3,070	20,800	10,800	25,200	5,600	7,000	3,150

* Mean of two 5 min. samples. † Trace obscured by smoke (count too low).

(3) *Changes in air spora at 7 cm. a.g.l. in barley during a 24 hr. period*

16–17 June 1953. In small plot of spring sown barley, Great Field II, Rothamsted Experimental Farm, Harpenden. During the whole period wind speeds were low; atmospheric humidity was very high with some mist.

Samples were taken at intervals of 6 hr. at the times shown in Table 3. The barley was heavily infected with *Erysiphe graminis*, and fungi belonging to the Sporobolomyceteae were abundant on the leaves. At each sampling occasion two consecutive 50 l. (5 min.) samples were taken on separate slides; these were scanned by making ten traverses across the trace. The counts obtained (Table 3) give the number of spores in 1·33 l. of air, and must be multiplied by 750 to give the number of spores per cubic metre.

Table 3. *Analysis of air spora at 7 cm. a.g.l. among barley at Rothamsted 16–17 June 1953*

Date ...	16 June				17 June					
Time (G.M.T.) ...	17.00		22.00		05.00		11.00		17.00	
Sample of pair ...	1	2	1	2	1	2	1	2	1	2
Spore type:										
Sporobolomyces	362	444	775	867	232	254	82	108	216	168
Tilletiopsis	67	66	258	213	96	98	66	51	146	112
Cladosporium	7	6	2	11	2	1	44	42	18	12
Erysiphe	6	2	2	0	2	0	22	12	112	2
Other types	14	12	16	18	7	5	5	8	8	3
Total	456	530	1052	1109	339	358	220	221	500	297

There is good agreement between the two samples on each sampling occasion (except for the *Erysiphe* (*Oidium*) counts on the last occasion: this can be explained by an omission to allow sufficient time before taking the sample for dispersal of spores disturbed while placing the impactor in position among the foliage). Changes in concentration of the different spore types during the 24 hr. are well shown, and agree with the findings of Hirst (1953) that the hyaline basidiospore types have maxima during the night, while *Cladosporium* and *Erysiphe* have maxima during the day. Conditions were too humid for free liberation of *Erysiphe* conidia. The presence of *Sporobolomyces* was confirmed both microscopically, and by cultures on filter-paper through which a sample of air was drawn at 17.00 hr. on 17 January. The type described as '*Tilletiopsis*' is indistinguishable microscopically from the basidiospores of *Tilletia*. However, there was no known source in the locality which could give such a high concentration of *Tilletia*, and as the spores were known from other tests to be of local origin, it is most likely that they also came from members of the Sporobolomycetae on the leaves.

(4) *Changes in the air spora during a 24 hr. period in woodland*

22–23 July 1953. Poundfield Heath, New Forest, Hampshire. (N.G. Ref.: SU 286044.)

To test the feasibility of studying changes in the air spora in a place where it would be difficult to operate a power-driven trap, four sites were chosen for sampling at 4 hr. intervals within a 30 m. radius in a patch of oak and birch wood beside the Blackwater River. A single 50 l. sample was taken at each site at each sampling time. After mounting, each trace was scanned under the oil immersion by twenty cross-traverses at 0·5 mm. intervals. The operation of sampling all four sites occupied approximately 30 min. on each occasion, and the times given in Table 4 are therefore within ± 15 min.; the sites were sampled in the order A to D.

The changes with time of day, and differences between the four sites are clearly marked. For interpretation of the findings with this type of ecological work intensive collecting and identification of organisms in the area is an essential.

(5) *Changes in the concentration of* Cladosporium *spores during instability rain*

17 July 1953. 10.37 to 11.47 G.M.T.; height 24 m. a.g.l. in open lattice tower in Meteorological Enclosure, Rothamsted Experimental Station, Harpenden.

There had been little rain during the few days before this set of observations (14 July, 4·06 mm.; 15 July, nil; 16 July, 0·76 mm.). On the morning of 17 July instability cloud developed, and immediately before the first shower the concentration of *Cladosporium* spores at 2 m. a.g.l. at the foot of the lattice tower was estimated at 32,000/m.^{3} (130,000/m.^{3} at 7 cm. a.g.l.). During the subsequent sampling period there were showers of rain separated by fine intervals. Rainfall was measured on a recording rain gauge about 50 m. from the foot of the tower, and was supplemented by notes on the time at which rain fell. Data shown in Fig. 3 indicate that there was a rapid increase in the *Cladosporium* spore concentration (also noted with *Botrytis*) during the showers.

The automatic trap was operating during this series of observations at a height of 2 m. a.g.l. about 200 m. from the foot of the tower. The trace shows a dense but narrow band of *Cladosporium* spores at the time

Table 4. *Number of spores per cubic metre of air in oak and birch wood, New Forest, 22–23 July* 1953

(Description of sampling sites: A, 120 cm. a.g.l. under canopy near edge of a zone (of about 20 m. radius) bare of ground vegetation, but surrounded by Pteridetum. B, 7 cm. a.g.l. vertically below A, in bare zone, above a thin layer of leaf mould overlying clay (not sampled at 13.00 hr. on 22 July). C, 7 cm. a.g.l. in Pteridetum (with *Lonicera*), 10 m. south of A and B. D, over Blackwater River. Impactor unit suspended about 10 cm. above water, 1 m. from bank, 60 m. east of sites A–C.)

Date	22 July			23 July			
Time (G.M.T.) ...	13.00	17.00	21.00	01.00	05.00	09.00	13.00
Basidiospores							
Site A	9,600 *a*, *b*	5,600 *b*	8,300 *b*	8,300 *b*	4,600 *b*	1,300	4,300
B	.	16,600 *b*, *a*	22,400 *b*, *a*, *c*	22,500 *b*, *a*, *c*	13,100 *a*, *b*	10,000 *b*, *a*, *c*	9,100 *b*, *a*
C	165,000 *b*, *d*, *a*, *c*	71,900 *b*, *c*, *a*	133,000 *b*, *c*, *a*, *e*	51,000 *b*, *c*, *a*	39,400 *b*, *c*, *a*	107,000 *b*, *a*, *c*	120,000 *b*, *e*, *a*
D	9,800 *b*	10,500 *b*, *a*	8,700 *b*	4,500 *b*	7,100 *b*	3,300 *b*	4,600
Fusiform and filiform ascospore-like types							
Site A	7,900 *f*, *g*	–	+	4,100 *g*	1,600	–	8,600 *h*, *g*
B	.	+	1,000	25,200 *h*, *f*	5,200 *f*, *g*	+	17,600 *g*, *h*, *f*
C	416,000 *f*	22,800 *f*, *g*	4,200 *f*	114,000 *f*, *h*, *g*	7,100 *h*	1,000	71,000 *h*, *f*, *g*
D	5,500 *f*, *g*	3,800	2,600	5,900	4,900 *g*	4,500 *h*	22,200 *g*, *h*, *f*
Cladosporium							
Site A	–	2,600	1,800	+	–	1,000	3,800
B	.	3,000	+	3,400	+	2,000	2,000
C	+	–	1,500	+	1,000	2,000	4,100
D	+	–	+	3,000	+	2,000	+
Total spores (including types not listed above)							
Site A	18,800	10,200	7,100	15,300	7,200	2,800	20,300
B	.	20,800	27,000	57,500	20,600	14,300	31,300
C	582,000	96,500	142,000	167,000	48,700	113,000	312,000
D	17,400	14,700	12,500	15,000	14,700	13,100	35,000
Rain and dew	Nil	Nil	Nil	Nil	(Dew on open heath none under trees)	Nil	Rain for past 3 hr.
Ground, etc.	Wet after rain	Drying	.	.		Dry	Wet
Air temperature (° F.)	64·0	62·7	57·0	51·5	53·7	62·5	58·5
Relative humidity (%)	87	78	91	98	92	77	92

Basidiospore and ascospore types occurring in concentration greater than 2/l. are indicated by letters *a*–*b*, arranged in descending frequency.

Symbols. *a*, *Russula* type; *b*, other hyaline agaric types; *c*, *Thelephora terrestris* type; *d*, *Sporobolomyces* type; *e*, coloured agaric type; *f*, minute hyaline of the showers, corresponding to a mean concentration for the hour of 83,000 spores/m.^{3}, but no doubt temporarily higher as the narrowness of the band shows the high concentration to have persisted for less than an hour. Thus both the portable trap at 24 m. and the automatic trap at 2 m. a.g.l. were affected during the passage of the showers by a dense cloud of *Cladosporium* spores, possibly associated with upward movement of a spore-rich layer from near ground level.

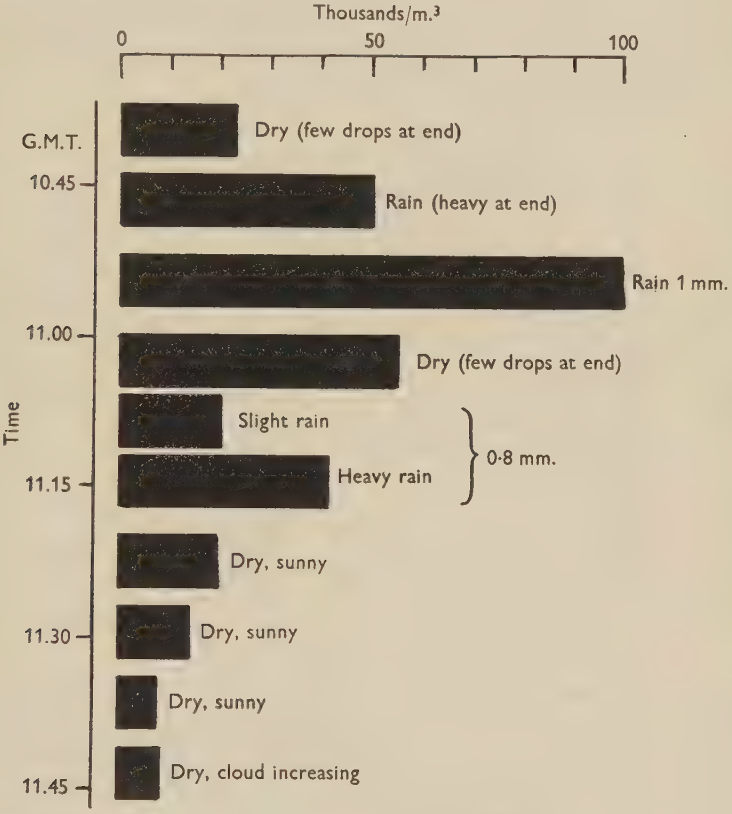

Fig. 3. Changes in concentration of spores of *Cladosporium* 24 m. a.g.l. during instability rain on 17 July 1953.

A similar increase of *Cladosporium* during rain has been reported by Ainsworth (1952) and Hirst (1953). For a better understanding of the phenomena it will be necessary to operate traps at several heights for short intervals during the onset of rain.

(6) *Spot samples*

The portable trap can easily be used to take 'spot samples' in places where it is of interest to determine the spore concentration of the air. Having made the estimate, however, in our present state of ignorance of the 'normal' air spora it is difficult to interpret a single isolated observation. The work of Hirst (1953) shows that at one site there are large changes both in spore concentration and in type at different times of day and in different weather. An isolated sample of 50 or 100 l. of air, taken during 5 or 10 min., cannot therefore give information capable of being extrapolated to give any general idea of conditions at that site. However, when a large amount of information has been accumulated from different places over a period of years from automatic traps and from systematic studies with the portable trap, it should become possible to recognize abnormal concentrations from a single sample. At the present time 'spot samples' are of interest in extreme conditions and in extreme environments. A few examples will show the kind of extremes met with during the first season's tests (Table 5).

Table 5. *Examples of 'spot samples' during* 1953

(No. of spores per cubic metre.)

Date and time	Place	Total spores	Main elements	
16 Apr., 12.45 hr.	Rocks covered *Xanthoria* and *Ramalina*, Samson, Isles of Scilly	Nil	Nil	
24 May, 08.50 hr.	Under oak, Geescroft Wilderness, Rothamsted	4,700	Hyaline basidiospores	3,000
			Cladosporium	600
31 May, 10.55 hr.	Bare downland, Juniper Top, Dorking, Surrey	1,700	Unidentified brown spore	1,100
25 June, 10.22 hr.	7 cm. a.g.l. in barley, Rothamsted Expt. Stn.	200,000	*Tilletiopsis*	146,000
			Cladosporium	20,000
			Sporobolomyces	17,000
8 Aug., 15.45 hr.	1·2 m. a.g.l. of wheat field, Crawley, Surrey	42,000	*Cladosporium*	25,000
			Pollen (nettle)	4,500
15 Nov., 11.28 hr.	1·2 m. grass pasture, Rothamsted Expt. Stn.	6,000	*Cladosporium*	2,300
			Basidiospores	1,500

(7) *Height above ground-level*

Because it is small the impactor unit can be used to sample air very close to the ground. Examples in Table 6 show that the zone within about 30 cm. of the ground normally has a much higher concentration of spores than the zone from 1 to 24 m. In the lowest metre the concentration gradient is very steep. More work on this aspect of the air spora is required, but it is already evident that in the first few centimetres above the ground the air is frequently rich in ascospores whose concentration decreases rapidly with increase in height. There is an indication that the concentration of basidiospores from agarics may decrease less rapidly with increase in height. During the warmer months a thin, spore-rich skin of air normally covers the ground where natural vegetation exists. Spores in the layers of the atmosphere above this are no doubt mainly derived from this ground layer by eddy diffusion.

Exceptions to the usually high spore concentration near the ground are themselves instructive. The air close to recently cultivated bare ground has been found relatively barren of spores; so also has the air under the canopy of a young cleanly cultivated potato crop.

Dense vegetation, while it may have its own sources of spores, often contains a 'private' air mass from which spores circulating in the atmosphere overhead appear to have been filtered out. This filtering action of vegetation may account for the low *Cladosporium* concentration under the tree canopy in the New Forest on 22–23 July. *Cladosporium* was also deficient in the air a few centimetres above ground level in a dense stand of *Pteridium* on Berkhamstead Common on 15 August 1953, where a sample showed 1900/m.^{3} under the canopy, as against 7100 and 9000/m.^{3} respectively in samples taken outside immediately before and after.

Table 6. *Spore content of air tested at different heights a.g.l.*

(Number per cubic metre.)

	Selected types					
Date, time and position	Height m. above ground-level	Basidiospores types per m.^{3}	Ascospores types per m.^{3}	Pollens per m.^{3}	*Cladosporium* per m.^{3}	Total spores and pollens per m.^{3}
26 July 1953, 11.40–12.42 hr., R.E.S. Met. Tower over cereals and other grasses	0·07	21,700	38,200	2,400	16,100	82,300
	1·0	1,000	700	1,200	9,000	14,700
	2·0	1,000	200	450	7,100	13,500
	6·0–24·0	400–800	100	75–450	1,500–6,000	9,400–13,800
15.46–16.50 hr.	0·07	9,000	4,100	1,000	11,200	30,600
	1·0	100	100	1,200	5,600	10,700
	2·0	400	100	600	11,600	18,300
	6·0–24·0	100–400	100–400	100–9,000	2,250–8,600	5,700–21,000
17 July 1953, 11.15–11.37 hr., R.E.S. Met. Tower	0·07	1,800	41,500	—	129,000	184,000
	2·0	1,100	100	—	32,000	39,000
	24·0	800	100	—	23,000	27,000
23 July 1953, 05.00 hr., oak birch wood, Poundfield Heath, New Forest, see Table 5	0·07	13,100	5,200	—	400	20,600
	0·3	14,500	3,000	—	100	19,000
	1·2	4,600	1,600	—	100	7,250
13.00 hr.	0·07	9,100	17,600	—	1,900	31,300
	0·3	8,300	11,800	—	1,500	24,200
	1·2	4,300	8,600	—	3,800	20,300
29 July 1953, 01.53–02.52 hr., R.E.S. Met. Tower	0·07	6,400	9,700	—	7,100	25,500
	2·0	8,200	6,700	—	400	18,000
	6·0	3,000	1,100	—	3,700	9,000
	12·0	2,200	1,500	—	1,900	6,700
	18·0	2,600	1,100	—	1,500	6,000
	24·0	3,000	100	—	400	3,000

(8) *Other sampling methods*

As a rule single samples are of little interest, but a comparison of two consecutive samples from slightly different positions, or the same position under different conditions, may give valuable information.

Other methods of using the portable trap which have been tested include: successive samples taken in a line across a mile of wood, heath and grassland; samples at different distances from a known source emitting spores; samples at one site taken first under undisturbed conditions and again while carrying out some operation such as grass mowing, or watering plants; or comparing conditions in a room and out of doors, within a short time interval.

For many of these purposes an adequate 'normal' sample is needed for comparison. This can sometimes be obtained by simultaneously running in the two different environments two impactor units, each controlled by its own plate orifice, from the one pump (the pump handle must then be turned at about 120/min.).

ERRORS

The portable spore trap is subject to a variety of errors which must be understood if valid deductions are to be drawn.

(1) *Error of sampling randomly distributed spores*

In the free atmosphere, remote from a source of spores, the distribution of spores through a small volume of air may be assumed to be random because of the diffusing action of atmospheric turbulence. A 50 l. sample would in any case be insufficient to give an accurate estimate of any spore type whose concentration was approximately 1 spore per 50 l., and with concentrations much lower than this the spore type would probably not appear in the sample at all. If the concentration is of the order of 1 or more randomly distributed spores per litre, a reasonable approximation should be obtained with a 50 l. sample. The portable trap is therefore inconvenient for measuring concentrations of less than 1000 spores/m.^{3}, without the excessive labour of long sampling times (though, with rests, 1000 l. samples could be taken to measure concentrations of somewhat less than 100 spores/m.^{3}). The portable trap is particularly convenient for sampling high spore concentrations.

(2) *Non-random distribution of spores*

Non-random distribution of spores may be due to two factors. Clumping of spores is common in *Cladosporium*, *Ustilago* and *Penicillium*, but rare in basidiospores, *Alternaria*, uredospores and in most but not all types of ascospores. In the former types the clump is the dispersal unit and at best the clump alone can be randomly dispersed.

The ground zone may usually be regarded as a mosaic of spore sources. In addition to the presence of randomly diffused spores from distant sources, air near the ground may contain small clouds of spores, coming from nearby sources, which have not yet had time to be spread out by eddy diffusion. A 50 l. (5 min.) sample might or might not sample one of these small clouds, and while recording faithfully the condition of the air which flowed past the impactor orifice during the sampling period, it could give an unrepresentative account of the air in the vicinity during a slightly longer period. The fact that the portable spore trap can give a short period reading may be an advantage for some purposes and a disadvantage for others. To get a sample representative of the air spora at a point near a strong, localized source over a longer time interval several methods are available: the sampling time can be increased; several successive independent samples can be taken at intervals; or, more economically, a mean value can be obtained by superimposing on one slide several samples taken with an interval of time between each.

(3) *Collection efficiency*

May (1945) has pointed out that for an accurate measurement of aerosol concentration the flow speed through an impactor orifice facing the wind must be approximately the same as the speed of the wind (sampling must be isokinetic). Errors due to non-isokinetic sampling rate will be small with small particles, but will cause appreciable errors with spores in the range 20–40 μ diam. As it is impracticable continually to adjust suction at the orifice to the wind speed, May (1945) has recommended using a standard velocity of 2·2 m./sec. through the orifice of the Cascade Impactor. In the Hirst trap and the portable trap described here the velocity through the orifice is 5·8 m./sec. and collection errors for larger particles would be expected to be greater than with the Cascade Impactor. Wind tunnel tests show that collection efficiency with the Hirst trap is below 100 %, varying from 55 to 90 % with *Lycopodium* (30 μ diam.) and from 5 to 80 % with *Ustilago avenae* (7 μ diam.) (Hirst, 1953). Separate wind tunnel tests have not been done with the portable trap, but as the tube of the impactor unit is slightly narrower, while other dimensions are the same as the Hirst trap, collection efficiencies would be expected to be slightly higher. The effect of collection errors is that the portable trap will in practice record too low, and under average conditions it probably collects only about 75 % of spores in the air sampled.

(4) *Retention efficiency*

Tested against the Cascade Impactor in the field it is evident that an impactor unit of the dimensions of the Hirst trap and the portable trap retain the smallest fungus spores with very high efficiency (Gregory, Hirst & Last, unpublished). This would be expected from the data given by Davies & Aylward (1951). The fine smoke particles, which are retained by stage 4 of the Cascade Impactor, are not retained by either the automatic or the portable traps, but over the size range of fungus spores errors from poor retention are probably negligible.

(5) *Scanning errors*

From the sample of 50–100 l. of air entering the orifice we may assume that all the spores are deposited on the trace. If the trace can be scanned completely it is possible to determine exactly the number of spores in the volume of air sampled. However, if high-power objectives are to be used in counting the smallest spores, several hours will be occupied in scanning the whole of a single trace. Usually therefore only a sample area is scanned, and this introduces another kind of error (irrespective of whether the air sampled was representative, or whether all the spores in that volume of air entered the impactor orifice). In practice ten or twenty traverses equally spaced across the whole of the long axis of the trace will be made under an 8, 4, or 3 mm. objective, and the total area scanned will be from 1/10 to 1/20 of the total area of the trace. The count must therefore be multiplied by 10 or 20 respectively to give the total number on the trace. Obviously a low count (0 or 1 for example) will give a very unreliable estimate of the total number of spores on the trace, while a count of 100 or more will allow the total to be estimated with reasonable accuracy.

(6) *Identification errors*

In the counts described here spores and pollen were classified into twelve to fifteen categories, with an 'unclassified' group for unknown, rare or inadequately seen spores. These categories have been united into larger groups for publication here, as many of the types were relatively infrequent

Errors or vagueness of identification are common to all types of spore trap which are scanned microscopically. Identification of a spore can seldom be carried to the species level with any high degree of probability and artificial groups have to be adopted. The visual method remains however, the only method of investigating the entire air spora, many components of which cannot be grown in culture.

CAPABILITIES AND LIMITATIONS

The advantages of the portable trap are that it is light in weight and is easily carried to inaccessible places where it can be worked by hand. It is cheap and easy to construct. The impactor unit is small and can if necessary be placed nearer to the ground or other surface than can the larger automatic trap. The time of taking a sample can be determined to within a few seconds.

Its disadvantages in comparison with the Hirst trap are the small samples taken unless great labour is expended. The Hirst trap records continually day and night in all kinds of weather. The denser deposit obtained with the Hirst trap makes counting easier, and the smoothing of short-term fluctuations which it gives could only be achieved by expenditure of an enormous number of man-hours with the portable trap.

The portable trap, however, is particularly suited to the measurement of high spore concentrations, to sampling marked concentration gradients especially in the first metre above ground level, and to circumstances when a sharp time differentiation is required. The two types of trap are perhaps analogous in their uses to a thermograph and a simple thermometer.

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AN INDIRECT METHOD FOR THE STUDY OF FUNGI IN THE SOIL

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(With 3 Text-figures)

The method described gives a measure of the vigour of fungal growth in a substrate, and was designed especially for the study of fungi in soil where the measurement of growth involves unusual difficulties. The technique depends upon microscopic measurement of the amount of mycelial growth produced in 24 hr. from inocula of an indicator fungus in the substrate under investigation. This value is an inverse relation of the length of lag, which is itself dependent upon the previous vigour of the protoplasm. Possible applications of the method are discussed.

Introduction

In rare cases the growth of fungal hyphae in non-sterile soil is macroscopically visible and may be followed without difficulty (Waksman, 1922; Nowell, 1923). In the majority of soils, however, hyphal growth is obscured by the substrate, and the results of growth studies under such conditions may become confused by ambiguities inherent in the methods used. The major objection to most methods in current use lies in the fact that the rate of linear spread of a fungus over or through a soil or agar substrate is a doubtful indication of the ability of that substrate to act as a growth medium, due to the adoption by many fungi of a characteristically spreading type of growth on media poor in nutrients. The intensity of sporulation of a fungus is also an unsatisfactory criterion of the capacity of a substrate to support fungal growth. Chesters (1948) and Hawker (1950) have noted that conditions unfavourable to vegetative activity may promote or stimulate sporulation in fungi.

Garrett (1946) found that the production and growth of mycelial strands of *Helicobasidium purpureum* from a disk of agar on which the fungus had grown was greater when the concentration of nutrients in the inoculum was high. The purpose of the present paper is to show that this fact is of general application, and that the amount of mycelium produced by a ungus from an inoculum may be used as an indicator of the level of available nutrients in the substrate forming the inoculum. The method described may thus be used to provide a measure of the ability of a soil to support ungal growth.

Method and treatment of results

The method used here was based on the direct isolation technique of Waksman (1916). Particles of a selected, untreated soil were placed under terile conditions on the surface of Czapek-Dox agar in Petri dishes. After incubation for 24 hr. at 25° C. the size of each particle and the greatest amount of linear mycelial growth given by it were measured microscopically using a 3·2:1 objective and a ×6 micrometer eyepiece. A conversion factor was applied to reduce the units of measurement to millimetres. In using this method it was found that the mycelium which gave the greatest growth from soil particles larger than 0·6 mm. had the same morphology in any one soil. Subcultures of these mycelia on further study proved to belong to the same species. This fact enabled other substrates to be inoculated with the organism and, after colonization by it, compared with the original soil using the method described. The media inoculated included Brown's agar (2 % agar), 3 % malt extract agar (2 % agar), tap-water agar (2 % agar) and soil sterilized by autoclaving at 121° C. for 30 min. During autoclaving the dishes containing the soil were wrapped in heavy paper to reduce loss of water.

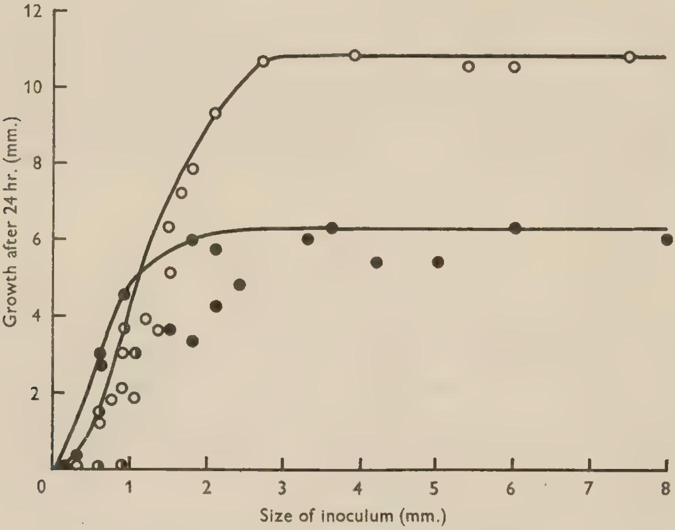

Fig. 1. Data from untreated soil (●) and from sterilized soil (○) showing maximum growth potentials.

In typical experiments twenty-four inocula—soil particles or small agar blocks—varying in size from 0·15 to 8·0 mm. were used for each substrate. These were placed four in each Petri dish. The readings obtained were plotted as inoculum diameter against the maximum linear mycelial growth after 24 hr. The soil particles used as inocula were regarded as approximating to spheres, whilst the agar inocula approximated in shape to cubes. In both cases the diameter varies as the cube root of the volume. For comparative purposes, therefore, the diameter gives a valid measure of the volume of the inoculum. Fig. 1 is a representative graph in which the data given by an untreated loam soil are compared with those given by the same soil sample after sterilization and colonization by the indicator fungus. It is seen that the points in the graph do not fall on a simple curve. However, a line can be drawn through the series of highest points given by each substrate. This curve indicates the maximum potential of the substrate to support growth in the indicator fungus. Similarly, if a line were drawn through the lowest points the minimum growth potential of the substrate would be shown. The area enclosed by these lines represents the variability of the substrate as expressed by this method. This variability may be due to the heterogeneity of the substrate or to irregular colonization of the substrate by the indicator organism. In order to compare more closely the results obtained from different substrates curves of maximum growth potential were used and were constructed as described.

It was found that for most substrates the static region of the maximum growth potential curve was reached at inoculum sizes between 2 and 4 mm. In only one case was an inoculum as large as 6·5 mm. required before this part of the curve was reached. On this basis and allowing a margin for safety, it was assumed that the increase in the amount of mycelium produced in 24 hr. had ceased at inoculum sizes lower than 7·5 mm. Also the degree of variability or scatter of the points was least in this static region of the curve. For these reasons, where it was desired merely to compare the maximum possible amount of mycelium produced by different substrates, six particles only, of sizes between 7·5 and 8·5 mm., were used for each substrate, the highest reading obtained from these being taken as the maximum value obtainable from that substrate under the conditions used.

Comparison of inocula from different substrates

A typical series of maximum growth potential curves given by inocula of different substrates with a single indicator organism is shown in Fig. 2. The soil used was an agricultural loam and the isolated organism was an unidentified *Mucor* sp.

The most obvious difference between the curves is in the height of the static part of the curve, and from the results of a number of experiments of this type certain generalizations may be made about this feature. As might be expected the two common laboratory media, Brown's agar and malt extract agar, always gave higher results than the other three substrates used. Tap-water agar had always a higher maximum growth potential than the untreated soil. Sterilization of the soil resulted in a marked increase in its ability to support growth. The increase was usually to a level above that of tap-water agar, but with some sandy soils the increase, although still marked, was to a level below that of tap-water agar.

Another feature of comparison in any series is the initial steepness of the curve. An investigation into the maximum growth potential curves of agar cultures of ages from 1 to 8 days was made. In these experiments the agar particles used as inocula were taken a standard distance from the centre of the growing cultures. The results show that the static part of the curve increased in height with age of the culture up to the third day, after which it remained constant. However, the initial steepness of the curve, which also increased to the third day, began to decrease slowly from the fourth to the eighth day, i.e. with older cultures a larger inoculum was required before the static part of the curve was reached. This indicates that increase in the hyphal density within the substrate occurs in the first 3 days and is followed by a gradual reduction, probably due to a loss in viability of certain of the hyphal branches resulting in a lower effective density of hyphae. The situation compares with that observed in the ageing of bacterial cultures as studied by counts of the number of viable cells in unit volume of the culture medium. The decrease with age in the initial steepness of the curve may then be regarded as senescence. The effective density of hyphae within the substrate, as indicated by the initial steepness of the curve, does not appear to be correlated with the greatest height attained by the curve (Fig. 2).

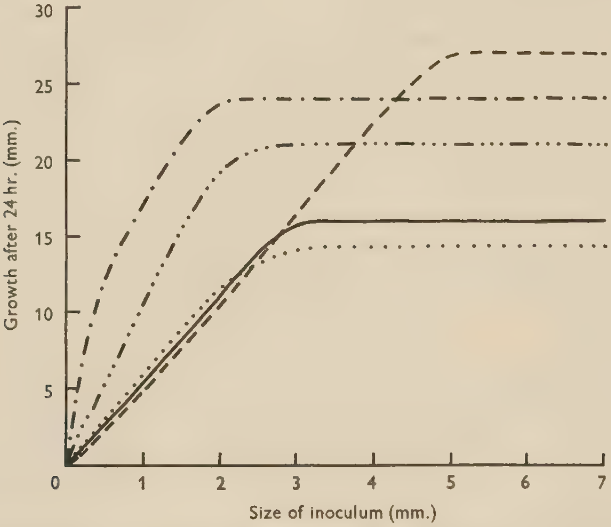

Fig. 2. Maximum growth potentials of different substrates (Brown's agar – – – –; malt extract agar –·–·–; sterilized soil ···–···–; tap-water agar ——; untreated soil ····).

It was noted that, in general, the degree of scatter of the points, i.e. the distance between the maximum and minimum growth potential curves, was smaller when the maximum growth potential was large. Thus those substrates which were better able to support growth also gave a more uniform colonization.

Examination of the method

In order to determine the reason for differences in the total amount of growth after 24 hr. from inocula of different types and sizes, the growth of mycelium from such inocula was followed in more detail than in the standard method. The agar particles used as inocula were cut 20 mm. from the margins of colonies of the *Mucor* sp. used in the previous section. The colonies had been growing for 4 days on plates of Brown's agar, malt extract agar, and tap-water agar respectively. Five inocula varying in size from 0·3 to 7·0 mm. were used for each substrate, and, after being transferred to poured plates of Czapek-Dox agar, were numbered and measured. The inoculated plates were incubated at 25° C., and measurements of the mycelium produced from each inoculum were made at 3-hourly intervals over a period of 24 hr. The results are presented graphically in Fig. 3.

An examination of this graph shows that the growth rate increased during the 3–6 hr. following the commencement of measurable growth. After this period a constant growth rate was maintained. This rate was virtually the same for all types and sizes of inoculum, a fact consistent with the growth of a given fungus on a standard medium. Thus the difference between the amounts of growth given by different inocula in 24 hr. appears to be due not to differences in growth rate but to differences in the duration of the lag phase. Hinshelwood (1946), in discussing a similar phenomenon in bacterial inoculations, found that a characteristic curve was obtained when the number of cells in the inoculum was plotted against the duration of lag. In the present study the inoculum size was plotted against the length of the lag phase, and curves similar to those given by Hinshelwood were obtained (inset to Fig. 3).

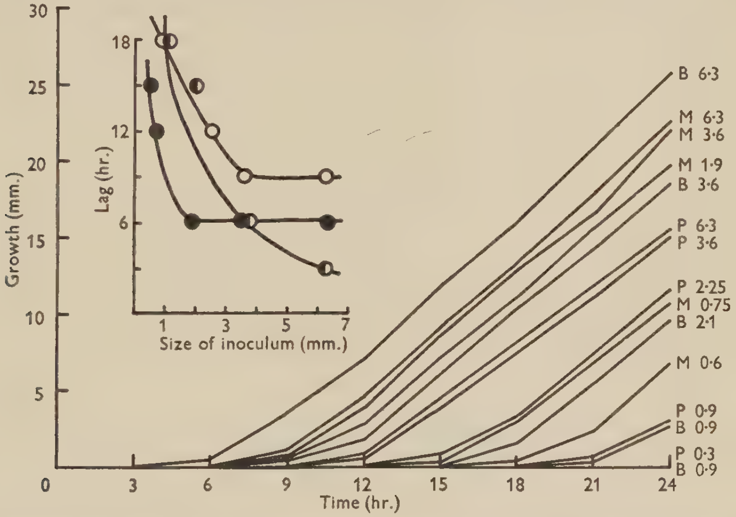

Fig. 3. Growth of mycelium from different types and sizes of inocula (B, Brown's agar; M, malt extract agar; P, tap-water agar; the figures refer to the diameter of the inocula in millimetres). Inset: relation between lag and inoculum size for Brown's agar (◐), malt extract agar (●), and tap-water agar (○).

Discussion

The value measured as the maximum growth potential of a substrate appears to bear an inverse relation to the length of lag of the indicator fungus in inocula of that substrate. Chesney (1916) regards lag as an expression of injury suffered by the inoculum in its previous environment. Further support to this view is given by Wilson & Miles (1946), who note that the lag phase appears to be a phase of rejuvenescence during which old but viable cells are acquiring the characteristics of young protoplasm. Hence, it would be expected that the lag would be longer when the protoplasm is farthest from a highly active state, i.e. under conditions least favourable for growth. Where the mycelium in the inoculum is vigorous the lag will be short, giving a high value for the maximum growth potential curve. The height of the stationary part of the curve, then, gives a direct measure of the vigour of the fungus in its previous environment.

A second factor to be read from the data given by the method is the initial steepness of the maximum growth potential curve. As the rise in this part of the curve is caused by the relation between inoculum size and lag, the point where the stationary part of the curve is reached may be expressed as the inoculum size above which no further decrease in the length of lag occurs. When this part of the curve is reached at a low inoculum size the fungal mycelium must be more concentrated within the inoculum than where a large inoculum is needed. The steepness of the initial part of the curve is therefore a criterion of the density of the mycelium within the substrate.

Of the aspects of the previous environment about which the method gives information the vigour of the fungus in the substrate is likely to have the widest application to studies of fungi in the soil. The danger of making comparisons between growth in sterile and in untreated soil is well known (Waksman, 1931; Garrett, 1938; Hessayon, 1953). The present method enables the investigator to discover what increase in growth potential occurs as a result of sterilization, and to allow for this. Where this increase is large then tap-water agar may be better than sterilized soil as an indicator of normal soil conditions. Another approach to soil mycological studies to which the method is applicable is in the investigation of the effect of different soil treatments on the growth of a fungus in the soil. If experiments were carried out along these lines using non-sterile soil the indicator fungus would need to be selected from those fungi growing out first from the inoculum. Alternatively, a medium selective to the fungus under observation might be used. Preliminary experiments have shown that in the mycelium growing out from a mixed inoculum it is possible to distinguish between the hyphae of certain species of fungi, for instance, those of *Mucor* and *Trichoderma*. This fact makes it possible to study the relationships between two or more fungi in soil as affected by environmental conditions.

I wish to express my thanks to Prof. C. W. Wardlaw for helpful advice and encouragement. This work was carried out during the tenure of a grant awarded by the Department of Scientific and Industrial Research to whom acknowledgement is made.

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SPORE PRODUCTION BY *HELMINTHOSPORIUM AVENAE*

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(With Plate 14 and 3 Text-figures)

Spores of *Helminthosporium avenae* are liberated into the air in similar numbers per unit of leaf area during primary (seedling blight) and secondary phases of leaf-spot disease of oats. No spores could be found in the surrounding air during the intervening period of disease-free growth. When spores were present in the air, they were found by night as well as by day; most were found at ground level, and numbers diminished with increasing height. There was no correlation between numbers of spores in the air and temperature, relative humidity and wind velocity.

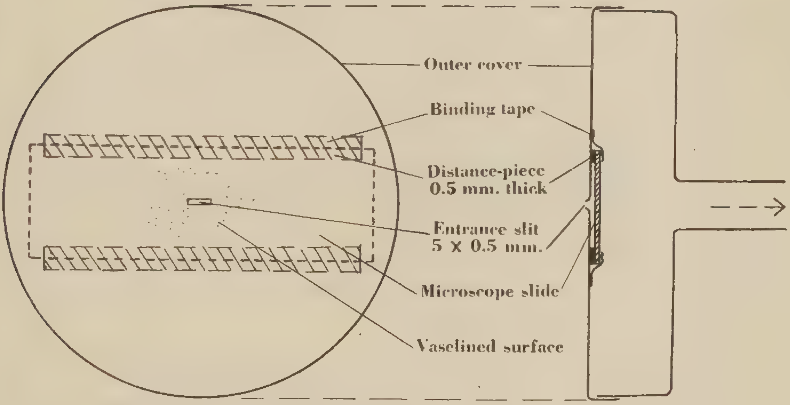
It is concluded that some factor depresses or inhibits the activity of the fungus in relation to the host when growth is free from disease. The factor does not operate earlier during primary disease, nor later at the secondary leaf-spot phase. Since the factor cannot be climatic, it is suggested that an investigation of conditions within the host plant itself is most likely to provide an explanation.

INTRODUCTION

The period of apparent health between the primary (seedling blight) and secondary phases of leaf-spot on the oat plant challenges the attention of plant pathologists. Leaves formed during the healthy period are without visible blemish from *Helminthosporium avenae* Eid. O'Brien & Dennis (1933), moreover, could not find mycelium in such tissues, though it was readily seen when parasitism was active during primary and secondary phases. Several writers (e.g. Turner & Millard, 1931) have stated that the secondary infection with *H. avenae* arises from spores produced on dead leaves of the primary phase. This would, indeed, seem to be the explanation, for no alternative host for the fungus is known. If, however, spores can be produced in sufficient quantity to initiate the secondary phase, what happens to them during the earlier healthy period? The present paper was planned to study the relative numbers of spores liberated into the air through the whole growth cycle of the oat plant. Periodical checks were, indeed, made throughout the whole year, and the main purpose was to see if spore liberation bore any relation to the three phases of primary disease, healthy growth and secondary disease.

METHODS

The numbers of *Helminthosporium* spores in the atmosphere were estimated by sucking air through an 'impinger' trap. This consisted of a microscope slide, lightly smeared in the centre with 'Vaseline' and held within a container, 0·5 mm. away from an orifice 5 × 1 mm. (Text-fig. 1). The trap was so arranged that air was sucked over the ends of the slide, thus giving a relatively long passage over the vaselined area. Suction was provided by an Edwards 'type IV' vacuum pump, and the exhaust air was pumped through an ordinary gas meter to measure the volume.



Text-fig. 1. The spore trap; optical plan (left) and vertical section (right). The tube shown by the arrow is connected to the suction pump.

Dr P. H. Gregory of Rothamsted Experimental Station kindly reported: 'I have now completed a set of runs to test the efficiency of your trap, using *Lycopodium* spores, dia. 33·1 μ in the wind tunnel. The volumes passing the trap were measured by a "Rotameter" and were kept at 4·75 l./min. Calibration was kept at this speed because it is approximately your normal rate of suction in the field:

Wind speed (m./sec.)	Approximate efficiency (%)
9·15	130
5·3	103
1·0	93

'The efficiency over 100 % is not unexpected. According to my calculation the velocity of intake through the orifice of the trap at 4·75 l./min. will be about 5 m./sec. So at speeds above this, spores are being literally blown into the hole by the wind, as well as being sucked in by the pump. At wind speeds below the speed through the orifice the trap will be acting as a bluff body and spores will tend to be carried around the trap in the streamlines and so the trap will tend to give a slightly low reading. I think all this really shows that over a wide range of wind speeds the trap as operated gives a result pretty close to the mark....'

The apparatus was mounted on a barrow (Pl. 1, fig. 1), and driven by electricity supplied through a long flexible cable, so that it could be operated within or near to an oat crop, variety Star, grown for the purpose. Routine estimations of seasonal distribution of spores were taken at the leeward edge of a crop and at a standard height of 1 ft. from the ground. Samples were, however, taken at different levels for special purposes.

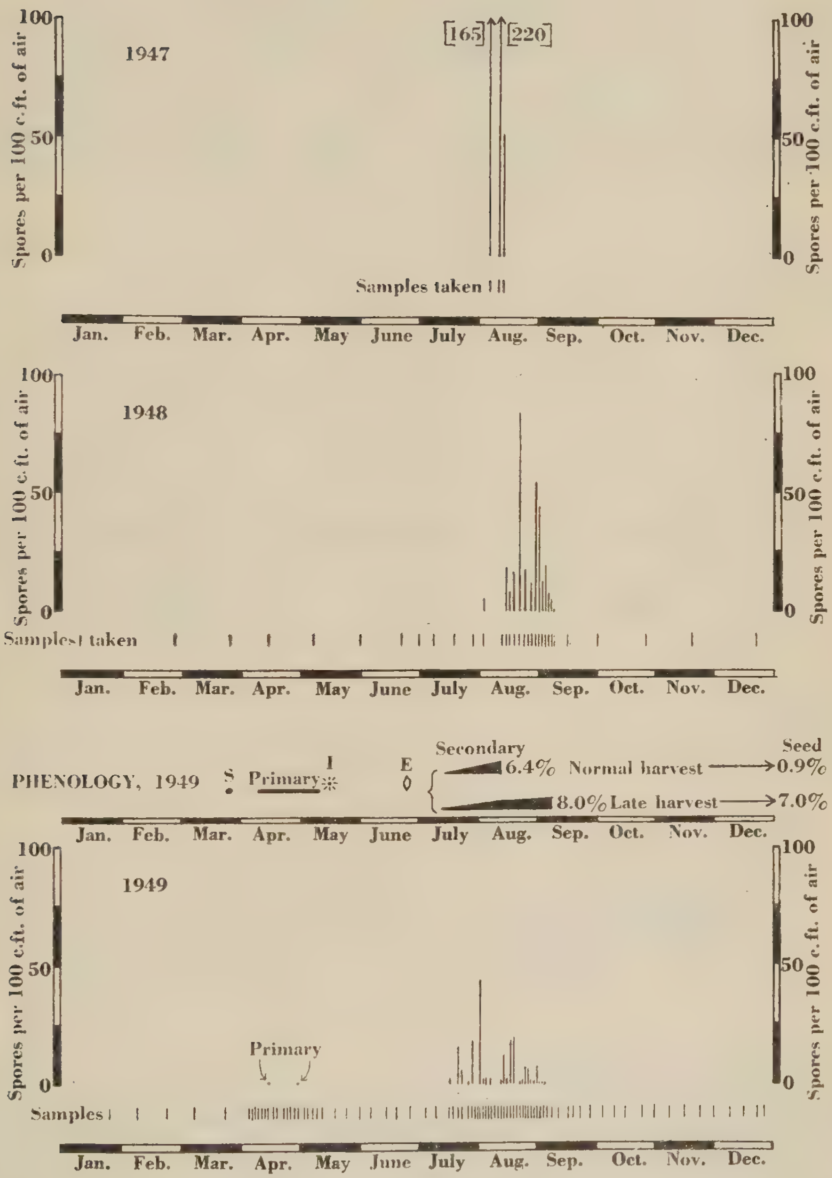
Some 200–300 cu.ft. of air were sucked through the trap for each determination; the slide was removed, a drop of lacto-phenol solution placed over the collection, and a cover-slip added. Examination was then made by systematic movement of the slide under a microscope by means of a scaled stage micrometer. *Helminthosporium* spores were easily recognized (Pl. 14, fig. 2). It is, of course, only strong presumption that they were *H. avenae*, but that they belonged to any other species is a somewhat remote possibility. Small plots of barley in the same field were not attacked by Net Blotch (*H. teres*) in 1948, and only to the extent of 0·1 % in 1949 and 2 % in 1947. The barley was at least 100 yards distant from the oat crop used for the determination, was rarely to windward of it, and was harvested before the *Helminthosporium* spores began to appear in quantity.

Screened wet- and dry-bulb mercury thermometers were whirled at various heights above ground during each estimation for spores, to measure temperature and relative humidity. Wind velocities were also measured by means of a sensitive cup anemometer.

Quantitative measurements of the severity of primary and secondary leaf-spot were those from the long-term disease phenology plots maintained at the Department of Plant Pathology, Auchincruive (Grainger, 1950). Twelve varieties of oats are each replicated four times in randomized blocks. The same stock of seed has been used over the last nine years, and plot sizes, manuring and management have also been standardized. Secondary leaf-spot is estimated by means of a 'standard area' diagram; primary leaf-spot by sowing fifty seeds in each of four pots of sterile sand during the winter months. The number of infected seedlings is expressed as a percentage of those which grow, and the percentage germination is also obtained.

Results

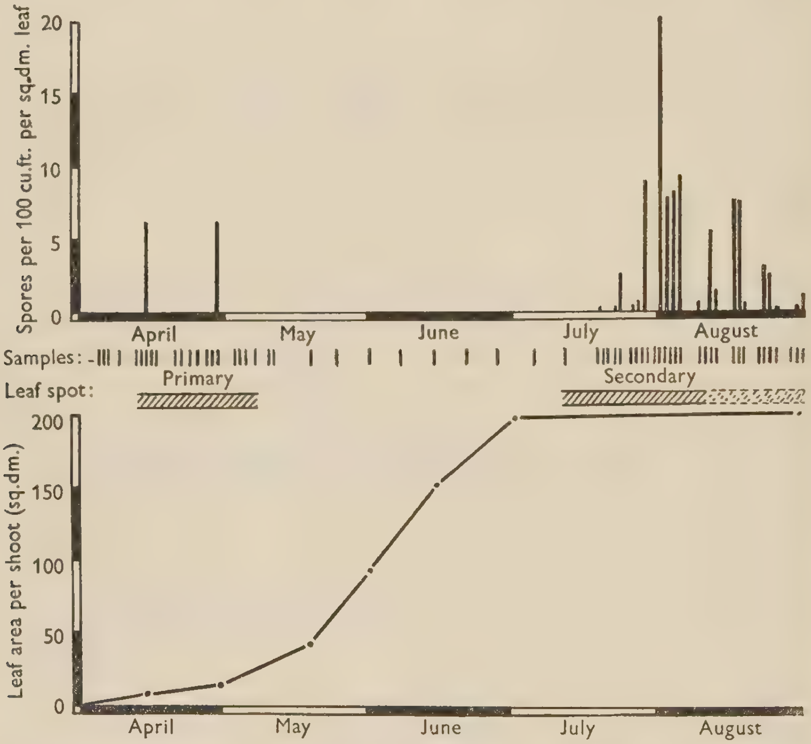
Text-fig. 2 shows the general results over a period of three years. Those for 1947 were little more than a test of methods during the secondary phase of disease. The numbers of spores recorded were, however, much higher than in the two following years. Periodic estimations were made through the year in 1948, with moderate numbers of spores at the secondary phase but none during primary disease. It was realized that sampling would have to be much more frequent. Estimations in 1949 were accordingly made at basic weekly intervals during the growing period and at 1- or 2-day intervals during the times of disease. Spores were then found during both phases of leaf-spot, but at concentrations less than 1 per 100 cu.ft. of air at primary phase. Leaves of the oat plant were quite small during this period, and it seemed necessary to relate the spore-load in the air to some standard of leaf area. Text-fig. 3 accordingly expresses the results for 1949 as the number of spores per 100 cu.ft. of air per sq.dm. of average leaf area per shoot. The intensity of spore production is then seen to be very similar during primary and secondary infections. The rising curve of leaf area during May and June (Text-fig. 3) emphasizes the absence of spores during this time. It would have become increasingly easier, as leaf area increased, to find them by the method employed. Not one spore, however, was found



Text-fig. 2. Seasonal distribution of *Helminthosporium* spores in the air near an oat crop, Auchincruive, with phenological data for 1949. S, time of seed sowing; I, flower initial formed; E, inflorescence emerged. 'Primary', 'secondary' and 'seed' refer to phases of the leaf spot fungus, as do the percentages.

from the beginning of May until after the middle of July. It seems fairly safe, therefore, to conclude that there is spore production during the occurrence of primary and secondary disease, but none, in measurable quantities, during the intervening healthy period, nor, indeed, at any other period of the year.

Table 1 shows the amounts of primary and secondary disease on twelve neighbouring oat varieties from 1946 to 1950. Comparison of these results with those shown in Text-fig. 2 does not suggest any close relation between the magnitude of secondary spore load and the amount of seed-borne primary disease in the following year. The heavy spore load of 1947 was followed by 3·93 % of primary seedling blight in 1948. Moderate secondary spore production in 1948 was followed by the highest average amount of seedling blight (9·46 %) of the period. Only the light spore load of 1949 was followed by a light primary infection (0·9 %) in 1950. Results of the Auchincruive disease phenology plots over the last nine seasons (of which Table 1 is a representative part) have, moreover, given no consistent relation between the amounts of primary and secondary disease in any one season, nor between the percentage of secondary disease and the percentages of seedling blight and of germination in the succeeding year.



Text-fig. 3. (Above) Spore-load calculated in relation to leaf area; (below) variations in average leaf area per shoot.

The time during which corn in the stook is exposed to infection by spores of *H. avenae* seems to determine the amount of seed-borne infection much more than does the aerial spore load. Part of a crop was harvested one month later than normal in 1949; its grain had 7 % of seed-borne leaf spot as against 0·9 % on grain harvested at the normal time. The amount of secondary leaf-spot with normal harvest (6·4 %) was, as would be expected, similar to that with late harvest (8·0 %). Text-fig. 2 shows this result under 'Phenology, 1949'.

Table 1. *Amounts of primary and secondary leaf-spot, with germination figures*

		1947			1948			1949			1950	
variety	1946 Sec. L.S. (%)	Germination (%)	Prim. seedling Bl. (%)	Sec. L.S. (%)	Germination (%)	Prim. seedling Bl. (%)	Sec. L.S. (%)	Germination (%)	Prim. seedling Bl. (%)	Sec. L.S. (%)	Germination (%)	Prim. seedling Bl. (%)
ellous	12·5	96	0·5	7·5	90	4·0	6·8	96	13·5	6·75	91	1·0
	15·0	96	0	7·1	74·5	2·0	5·4	97	3·0	4·75	97	1·0
ard	16·5	96	0	6·8	78	4·0	5·9	96·5	8·5	5·75	97	1·0
o	14·0	97·5	1·5	6·6	91·5	2·0	5·3	98	18·5	8·75	97	0·5
ance	6·5	81·5	1·5	9·4	67·5	2·5	5·6	96·5	5·5	7·25	97	0·5
ry	6·0	89·5	1·0	7·9	90	3·0	6·2	95·5	15·5	18·25	96	2·5
ounty	7·5	89·5	0·5	7·9	92·5	6·0	6·1	97	9·5	5·25	91	1·0
er	16·0	93·5	0·5	7·4	89·5	3·0	6·4	98	8·5	5·75	97	0·5
nando	3·0	99	0	9·1	91·0	1·5	6·3	97·5	2·5	5·75	97	1·5
re	19·0	97·5	0	9·5	91·0	6·5	7·3	98	7·5	7·75	97	0
Miller	11·0	98·5	0	10·5	88·5	7·0	5·2	97	8·0	4·75	98	0·5
Scott	20·0	96·5	0	9·8	93·0	5·5	6·8	97	13·0	6·25	98	1·0
ge, all ieties	12·3	94·3	0·46	8·3	86·4	3·93	6·1	97·0	9·46	6·4	96	0·9

ures for the percentage leaf-spot in each year are the averages of four plots of each variety. Percentages mination and of primary seedling blight are averages from four pans, each with fifty seeds.

Table 2. *Numbers of* Helminthosporium avenae *spores at various heights above ground*

Averages of twenty readings, 5–12 Aug. 1947, Auchincruive.

	Spores per 100 cu.ft.	Temperature (° F.)	Relative humidity (%)	Wind velocity (ft. per min.)
Ground level	399	59·5	78	1
2 ft.	134	66	68	12
4 ft.	84	65	66	37
6 ft.	96	64	70	54

Spores were found by night as well as by day. Most spores were found near the ground within an oat crop, and the numbers diminished with ncreasing height (Table 2). This diminishing spore load seems to be at irst sight somewhat related to relative humidity and wind velocity Table 2). It was, however, possible to test this indication in a much more atisfactory manner by comparing relative daily spore loads with their ppropriate temperatures, relative humidities and wind velocities during econdary spore production. Some sixteen estimations were made in 1948 nd double the number in 1949. All the readings of meteorological factors and spore load were made 1 ft. above the ground, and the statistical comparisons are given in Table 3. They show a complete lack of correlation between the numbers of spores in the air and any of the meteorological factors mentioned. The greatest concentration of spores at ground level shown in Table 2 must, therefore, represent the natural tendency for relatively heavy spores to fall downwards in the air. Spores at a height of 6 ft. above ground level would be carried there temporarily by wind turbulence. It would be natural to expect lower numbers above the crop than within it.

Table 3. *Lack of correlation between* Helminthosporium avenae *spore content of the atmosphere and meteorological factors*

	1949		1948	
Correlation*	Correlation coefficient r	Probability by t-test P lies between	Correlation coefficient r	Probability by t-test P lies between
1:2.34	−0·169	0·5 and 0·4	−0·311	0·6 and 0·5
1:3.24	−0·303	0·2 and 0·1	+0·250	0·5 and 0·4
1:4.23	−0·066	0·8 and 0·7	+0·079	0·5 and 0·4

* 1 = number of *Helminthosporium* spores per 100 cu.ft. of air.
2 = percentage relative humidity at the time of spore estimation.
3 = wind velocity in ft. per min. at the time of spore estimation.
4 = temperature in ° F., at the time of spore estimation.

1:2.34 means a correlation coefficient obtained by the method of partial correlations between spore content (1) and relative humidity (2), with wind velocity (3) and temperature (4) held constant.

Discussion

The main result of this investigation is that spore production by the fungus *H. avenae* is very greatly reduced or non-existent during healthy growth of the oat between the spore-producing primary and secondary phases of leaf-spot disease. This fits well with the absence of visible symptoms, and also with lack of mycelium in the tissues during healthy growth contrasted with its presence at both phases of disease (O'Brien & Dennis, 1933). All these results suggest that some factor has depressed or inhibited the activity of the fungus in relation to the host when plant growth is healthy. This factor does not operate in earlier nor in later growth. In view of the demonstrated lack of correlation between spore liberation and temperature, relative humidity and wind velocity (Table 3), the factor could hardly be the result of variations in general climate. It is, however, still difficult to conceive that micro-climate round the disease lesions could have no effect. The present results as expressed in Text-fig. 3 nevertheless show that it is just as easy to obtain similar aerial spore loads per unit of leaf area in April (primary disease) as it is in July (secondary phase). The differing climatological factors for these two months in 1949 are given in Table 4, and, when compared with Text-fig. 3, reinforce the conclusion that general climate does not affect spore liberation.

The only likely factor as yet uninvestigated seems to be the possible effect of the host plant itself. Determinations already made in this Department show that there are indeed considerable variations in the growth and

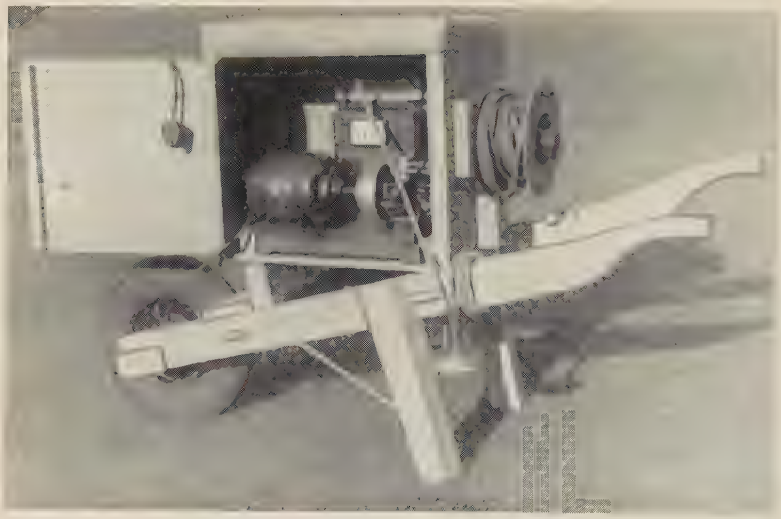


Fig. 1

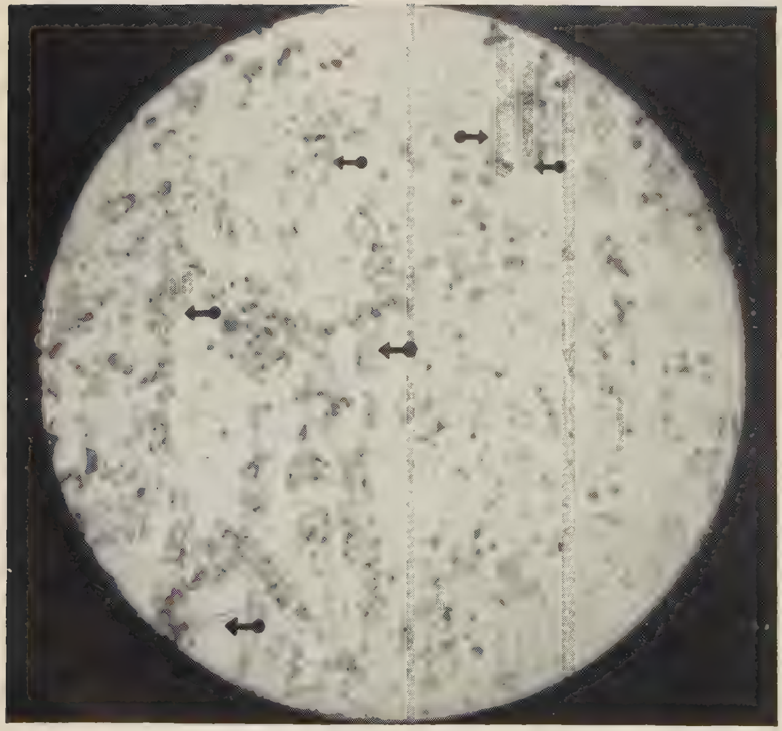


Fig. 2

Table 4. *Average climate in April and July*, 1949, *at Auchincruive*

	Temperature (° F.)	Rainfall (in. per month)	Bright sunshine (hr. per month)	Average % R.H. at 9 a.m.	Wind speed, average per month (m.p.h.)
April	47·9	1·83	132·8	79·4	13·2
July	59·4	2·59	167·6	77·7	6·5

composition of the oat plant. These occur at times which suggest that they could be invoked to explain the occurrence of healthy growth between two periods of active disease. The results will, however, be combined with those from other disease-host relationships for a separate paper.

The help of Miss Moira C. Thomson with the field determinations is gratefully acknowledged.

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EXPLANATION OF PLATE 14

Fig. 1. The spore trap and its portable mechanism. The trap itself hangs from the rubber tubing at the near-side barrow leg; the meter is behind, in the box, and the suction pump (right), with its motor (left), is in front.

Fig. 2. *Helminthosporium* spores (shown by the arrows) on the spore-trap slide.

(*Accepted for publication* 24 *February* 1954)

OCCURRENCE OF AQUATIC HYPHOMYCETES UPON THE ROOT SURFACES OF BEECH GROWN IN WOODLAND SOILS

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Clathrosphaerina zalewskii van Beverwijk and *Varicosporium elodeae* Kegel have been isolated from the root surfaces of beech seedlings grown in beechwood soils. Previous isolations have been recorded from submerged leaves in fresh water. It is suggested that aquatic hyphomycetes may be active in various soil sites.

As has been suggested before by Chesters (1949), Garrett (1951) and other soil microbiologists, it would be worth while developing selective methods of isolating groups of fungi that might be active in various soil habitats.

Harley & Waid (unpublished) have sampled the fungi that are active on the surfaces of the roots of the beech tree by washing roots in repeated changes of sterile water. A standard technique has been evolved which ensures the removal of fungal propagules from the root region and often enables the mycelia of fungi which grow slowly in culture to colonize nutrient agar media. Many forms of phycomycetes, imperfect fungi, sterile mycelia, some basidiomycetes and two species of aquatic hyphomycetes have been isolated by this method.

In the studies reported here phials with screw-top caps, containing roots and 10 ml. of sterile water, were shaken at the rate of 300 beats per min. for 20 periods of 2 min. Between each shaking period the water was decanted away and a fresh 10 ml. of sterile water was added to the phial. The washed roots were cut into 0·2 cm. lengths and were plated on nutrient agar and incubated in the dark at 25° C.

The two imperfect fungi, *Clathrosphaerina zalewskii* van Beverwijk (1951) and *Varicosporium elodeae* Kegel (Ingold, 1942), have been isolated from the roots of beech seedlings which had been grown in beechwood soils but not from the roots of seedlings which had been grown in a nursery garden soil.

On the first occasion *Clathrosphaerina zalewskii* was observed it occurred three times on portions of a beech root collected in April 1952 from the litter layer of the beech stand in Bagley Wood, Berkshire. The number of times aquatic hyphomycetes have been observed growing from washed portions of beech root which have been plated on to the medium of Lochhead & Chase (1943) is shown in Table 1.

Previous isolations of these fungi have been reported to have been made from the submerged leaves of broad-leaved trees and shrubs that were decomposing in fresh water (Ingold, 1942, 1943*a*, *b*; van Beverwijk, 1951). Bessey (1939) isolated *Varicosporium elodeae* from soil, and it was regarded

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Table 1. *Records of aquatic hyphomycetes observed on the root systems of beech seedlings grown in pots containing soils collected from beechwoods*

Date sampled	Source of soil	Region of root	Number of records		Number of plants on which fungus occurred
			V. elodeae	*C. zalewskii*	
27. v. 52	Bagley Wood	Tap	—	13	2 out of 5
17. vi. 52	Bagley Wood	Tap	—	8	5 out of 5
27. v. 53	Bagley Wood	Tap	—	1	1 out of 5
1. vii. 53	Bagley Wood	Laterals	—	9	2 out of 5
4. vi. 52	Shotridge Wood	Tap	2	—	1 out of 5
20. viii. 52	Shotridge Wood	Laterals	6	1	One seedling in each sample
25. viii. 52	Shotridge Wood	Laterals	1	7	
26. viii. 52	Shotridge Wood	Laterals	6	2	
Total			15	41	

by him as an uncommon fungus of soils. Prof. C. T. Ingold has drawn attention (personal communication) to an observation of Scourfield (1940) who found spores of *Tetracladium setigenum* Grove (wrongly identified as *T. marchalianum* De Wild.) in woodland leaf litter. The two species observed on beech roots are poor competitors on nutrient agar media and are soon overgrown by other fungi on the isolation plates. They do not demonstrate any striking cultural characteristics and can only be identified by the nature of their propagules, so they could easily be overlooked on crowded isolation plates. It is possible, however, that aquatic hyphomycetes are active on or in many other sites in soils other than the roots of the beech tree.

The author carried out the work reported here under the supervision of Dr J. L. Harley during the tenure of an Agricultural Research Council Training Grant. He is indebted to Prof. C. T. Ingold for his kindness in drawing attention to the observation of D. J. Scourfield.

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A LEAF DISEASE OF THE OIL PALM (*ELAEIS GUINEENSIS*) CAUSED BY *HELMINTHOSPORIUM HALODES* DRECHSLER VAR. *ELAEICOLA* VAR.NOV.

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(With Plate 15 and 2 Text-figures)

Symptoms

During a visit to the oil palm plantation of Elisabetha in January 1953, a hitherto undescribed leaf disease of nursery palms was observed. The damage sustained by the infected palms was considered sufficiently serious to warrant investigation. More recently, palms with a mild infection of the disease have been seen in the nurseries of the Yaligimba plantation.

The disease has only been observed in nursery palms in the height range of 25–90 cm. At Elisabetha approximately 2 % of the palms were badly infected and had to be removed. First symptoms of the disease are usually found on either of the two youngest, open leaves or the closed, 'spear' leaf. Small pale green spots are found scattered over the leaf laminae. These spots enlarge, become yellow-coloured and have a pin-point brown spot at their centres. Further enlargement of the spot takes place and the yellow colour gives way to a pale brown and still later a dark brown colour. On the upper surface of the leaves the spots are slightly zoned with the centre of the spots being more red brown than the remainder. In the final stage, the spots are usually oval-shaped with the upper surface slightly sunken and the lower surface a paler brown than the upper surface. The size of the spots varies between 1·5 × 1 mm. and 5 × 3 mm. The tissue immediately adjacent to the spots is chlorotic and the coalescence of neighbouring spots occurs frequently.

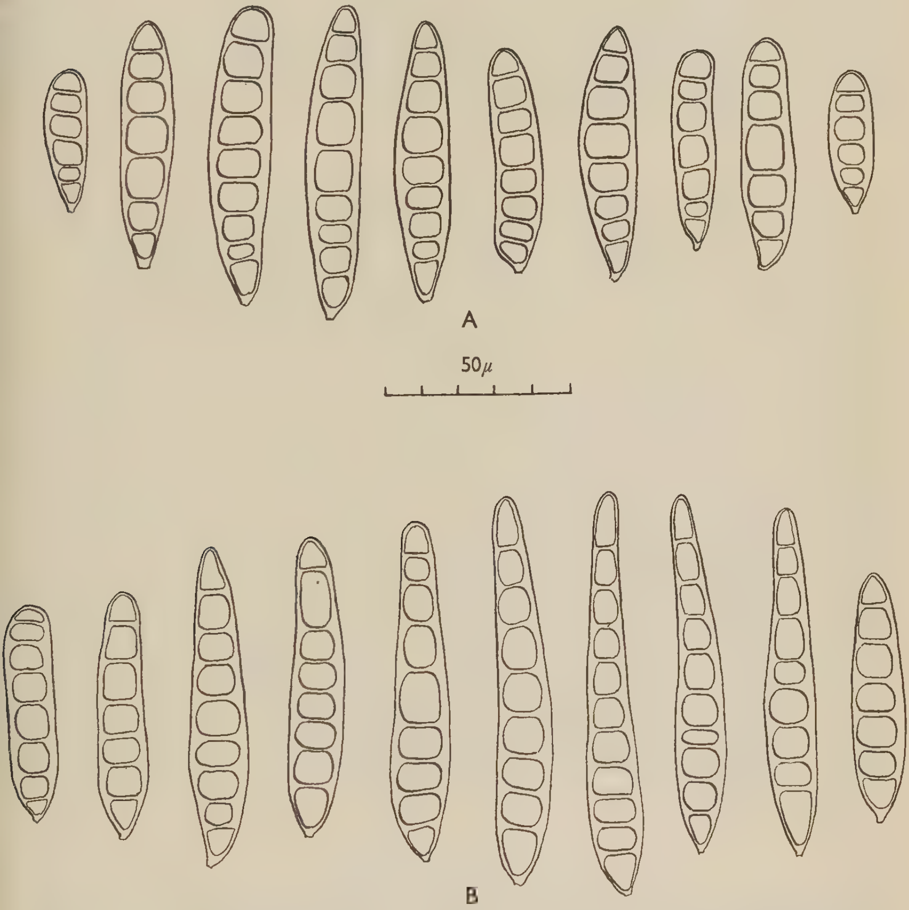
With a heavy infection, large areas of the leaf laminae dry out and become a grey-brown colour with the dark brown spots retaining their identity in the desiccated tissue. Under such conditions the emerging 'spear' leaves become heavily infected before opening, and the diseased areas of these leaves are not confined to spots but include considerable parts of the leaf laminae. At the time of opening, these leaves are highly chlorotic with ill-defined spots and are extensively rotted. Conidia are only rarely found in association with the diseased spots, but may be found in profusion on fairly recently rotted parts of heavily infected leaves. In such cases the conidial mass can be seen as a sooty layer with the naked eye, and when examined microscopically they can be identified as conidia of a *Helminthosporium* which is here proposed as a new variety of *H. halodes*.

Helminthosporium halodes Drechsler var. **elaeicola** Kovachich, var.nov. (Pl. 15, Text-fig. 1)

A typo ita differt: conidia in statu naturali elliptica vel obclavata. In cultura ut typus.

Habitat in foliis *Elaeis guineensis*, Elisabetha, Belgian Congo, June 1953, W. G. Kovachich (Herb. I.M.I. No. 52930 a typus).

Colonies amphigenous. Mycelium immersed in the substratum; hyphae hyaline to pale brown, 3–5μ wide. Conidiophores single or in small groups (2–4), emerging through the outer epidermal walls, straight or flexuous, sometimes geniculate, brown, pale at the apex with one or more well-defined scars, 25–150μ long, 5–8μ wide, with a swollen basal cell 12–17μ wide. Conidia straight or slightly curved, elliptical to obclavate, olive brown, 33–125μ long, 10–18μ wide, with a protruding basal hilum 2–3μ wide, 4 to 12-septate, the end septa sometimes accentuated.



Text-fig. 1. Conidia of *H. halodes* var. *elaeicola*. A, from culture; B, from infected leaf.

The fungus is readily isolated from diseased leaves and grows well on malt agar, potato-dextrose agar, cornmeal agar and palm-leaf extract agar. Sporulation is abundant on all these media. In culture the conidia are cylindrical to elliptical, tapering slightly at both ends but never becoming obclavate. They are 32–96μ long, 11–16μ wide and 5 to 10-septate. The end segments are delimited by strongly accentuated septa and each conidium has a protruding basal hilum. Mature conidia germinate typically by the production of two polar germ tubes, produced at thin-walled regions at the apex and immediately adjacent to the hilum.

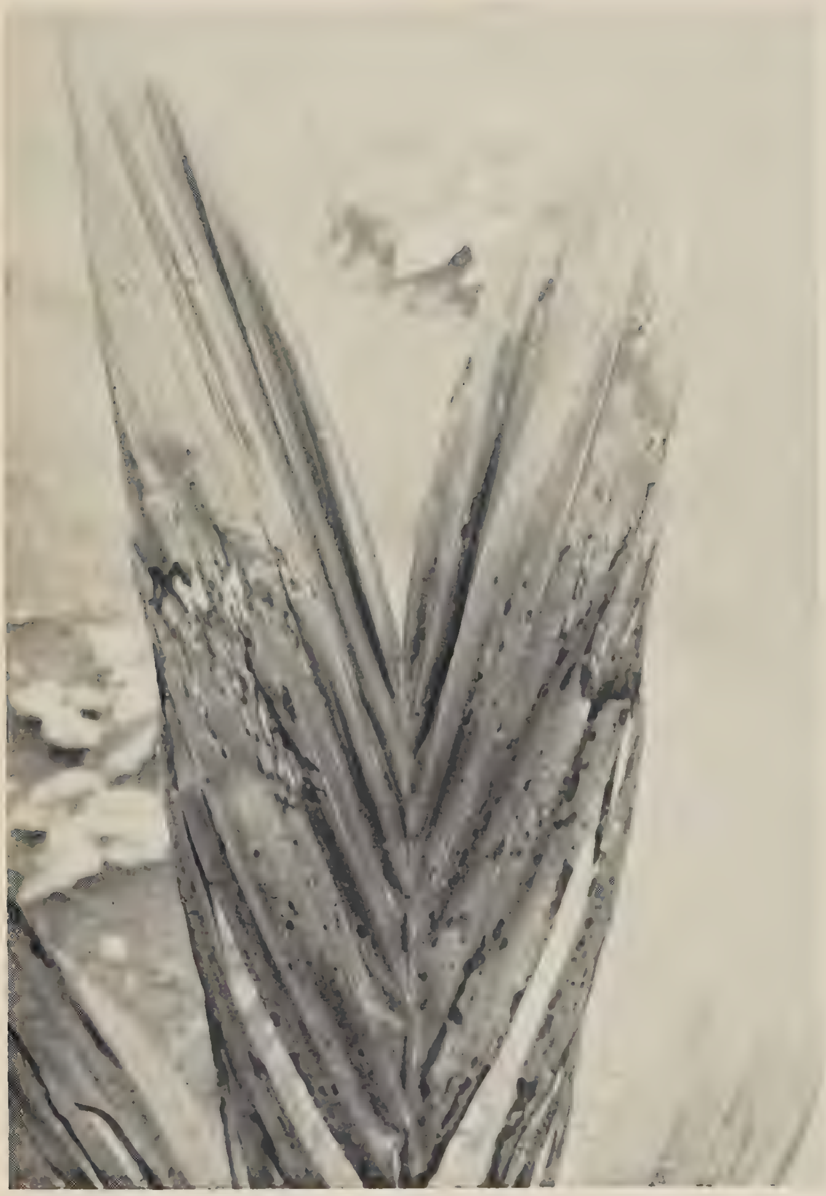
Although indistinguishable from *Helminthosporium halodes* in culture the production of obclavate conidia on the host serves at once to distinguish the variety. *H. rostratum*, another species in which the end septa are often accentuated, produces numerous rostrate conidia which are larger than those of *H. halodes* var. *elaeicola*. In *H. halodes* var. *tritici* Mitra the conidia are cylindrical or elliptical and up to 20μ wide.

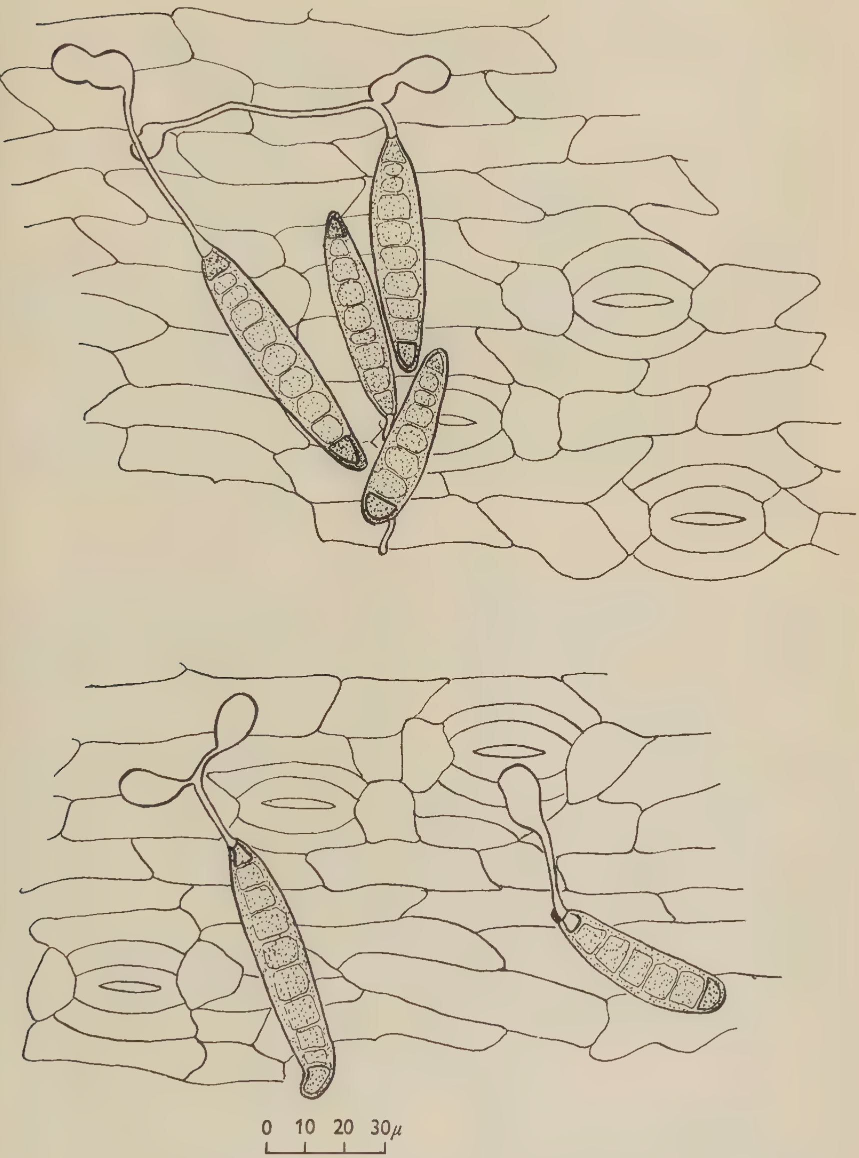
PROOF OF PATHOGENICITY

A concentrated spore suspension was obtained by adding sterile water to 10-day-old, pure cultures of *H. halodes* var. *elaeicola* on malt-agar slopes and agitating the tubes. The spore suspension was then painted on: (*a*) young leaves; (*b*) old leaves; (*c*) the upper surface of young leaves; (*d*) the lower surface of young leaves of palms of approximately 50 cm. height. Five palms were employed for each of the above treatments. A further five palms of the same size, ten palms of approximately 120 cm. and two palms of 300 cm. were sprayed with a spore suspension. Fifteen small palms, ten medium-sized and two large palms were maintained as controls. All the inoculated palms developed symptoms of the disease and all the control palms remained free from infection. Both spraying and painting with a spore suspension produced a very heavy infection of the young leaves of the small palms treated, and infection took place through both the upper and lower leaf surface. The old leaves of the small palms became only mildly infected whilst with the medium-sized palms, the typical spots only developed on the distal pinnae of the youngest open leaves. The taller palms failed to show typical spotting but faint, yellow, spotting of the pinnae of the youngest open leaf resulted from the inoculation. The disease symptoms were apparent 6 days after inoculation. *H. halodes* var. *elaeicola* was re-isolated from the diseased parts of the inoculated leaves. Pl. 15 illustrates diseased leaves resulting from inoculation.

SPORE GERMINATION

Young leaves of young palms were painted with a spore suspension of *H. halodes*, and samples of these leaves were taken at intervals to study the germination of the conidia. The leaf samples were cleared in lactic acid and stained with lacto-phenol cotton blue before examination. It was found that the conidia germinated and produced appressoria within 24 hr. Germ tubes were produced from either or both of the terminal cells of the conidia. Occasionally the germ tubes branched before forming appressoria (Text-fig. 2). Extremely few conidia failed to germinate.





Text-fig. 2. Germination of conidia of *H. halodes* var. *elaeicola* on a leaf of the oil palm.

Thanks are due to Dr M. B. Ellis for help with the taxonomy and to Huilever S.A. for permission to publish this report.

EXPLANATION OF PLATE 15

Leaves of the oil palm artificially inoculated with *H. halodes* var. *elaeicola*.

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STUDIES IN THE GROWTH REQUIREMENTS OF INDIAN FUNGI

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Twenty-six isolates of fungi from different host plants were tested for vitamin requirements. Eleven isolates (including several morphologically similar isolates of *Pellicularia koleroga*) were heterotrophic to thiamine, but their capacity to synthesize this vitamin was not completely lost as they do so from a mixture of thiazole and pyrimidine or from pyrimidine alone. *Piricularia oryzae*, however, was unable to do this in young cultures, but with advancing age utilized an equimolar mixture of thiazole and pyrimidine or pyrimidine alone. Some isolates were also tested for growth response with riboflavin, biotin and pantothenic acid. A strain of *Aspergillus niger* isolated from Indian soil was more sensitive in detecting traces of copper and molybdenum than the standard *A. niger* 'M' strain used for bio-assay of heavy metals.

Growth factors

Numerous investigators have shown that many fungi are unable to grow normally on strictly synthetic media unless minute amounts of one or more growth substances are supplied (Robbins & Kavanagh, 1942; Schopfer, 1943; Fries, 1948; Hawker, 1950; Lilly & Barnett, 1951). Thiamine deficiency is the most common among filamentous fungi, but not all thiamine heterotrophic fungi require the entire thiamine molecule; thus *Phytophthora* spp. require an external supply of intact thiamine for their growth (Robbins, 1938), while *Phycomyces blakesleeanus* (Schopfer & Jung, 1937; Robbins & Kavanagh, 1937; Sinclair, 1937) and *Torula* spp. (Robbins & Kavanagh, 1938*a*) grow if supplied with both the intermediate fractions, pyrimidine and thiazole. Other fungi like *Rhodotorula rubra*, *Parasitella simplex*, *Dematium nigrum* (Schopfer, 1937, 1938), *Torula* spp., *Pythium butleri*, *Sclerotium rolfsii* (Robbins & Kavanagh, 1938*a*, *b*, *c*) and *Tricholoma* spp. (Norkrans, 1950) require only pyrimidine, while growth of *Mucor ramannianus* (Mueller & Schopfer, 1937) is promoted by thiazole alone.

In this laboratory a number of fungi isolated in India were tested for their vitamin requirements by K. T. Mathew and S. Suryanarayanan with results shown in Tables 1 and 2.

Most of the fungi listed in Table 2 as showing vitamin deficiency were investigated in detail.

Corticium microsclerotia (Matz) Weber

Mathew (1953) reported that *C. microsclerotia* on a glucose nitrate medium failed to grow without a trace of thiamine, thus indicating its thiamine heterotrophic nature. The fungus had no partial deficiency for any of the growth substances tried other than thiamine.

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* Paper read to the 8th International Botanical Congress, Paris, 1954.

Table 1. *Indian fungi showing no vitamin deficiency*

Fungus	Source
Tolyposporium ehrenbergii	*Sorghum vulgare*
Diplodia sp.	*Citrus* sp.
Pestalotia sp.	Tea
Colletotrichum graminicolum	*Sorghum vulgare*
C. falcatum	Sugar-cane
C. capsici	*Capsicum annuum*
Gloeosporium sp.	Rubber
Pythium sp.	*Carica papaya*
Helminthosporium sp.	*Sorghum vulgare*
Fusarium vasinfectum	Cotton
Macrophomina phaseoli	Cotton
Curvularia sp.	*Cajanus cajan*
Fusarium udum	*C. cajan*
Neocosmospora vasinfecta	*C. cajan*
Phoma sp.	*C. cajan*

Table 2. *Indian fungi showing vitamin deficiency*

Fungus	Source
Sclerotium oryzae	Rice
Piricularia oryzae	Rice
P. setariae	*Setaria italica*
P. zingiberi	*Zingiber*
Piricularia sp.	*Eleusine coracana*
Piricularia sp.	Wheat (*Triticum vulgare*)
Piricularia sp.	Water grass (*Brachiaria*)
Ceratostomella sp.	*Cocos nucifera*
Phytophthora arecae	*Areca catechu*
Pellicularia koleroga	Coffee
(*Corticium microsclerotia*	Obtained from C.B.S. Baarn)

In the presence of an optimum amount of thiamine the fungus grew rapidly producing numerous sclerotia. The optimum amount of thiamine necessary for rapid and best growth was 2·0 μg./20 ml. of medium. Like many other thiamine heterotrophic fungi (Schopfer, 1937, 1938; Robbins & Kavanagh, 1938*a*, *b*, *c*; Norkrans, 1950) the capacity to synthesize this vitamin is not entirely lost by *C. microsclerotia*, and it was able to complete the synthesis of thiamine either from a mixture of thiazole and pyrimidine or from pyrimidine alone. However, growth with pyrimidine alone was very slow and not comparable to that obtained with the pyrimidine-thiazole mixture or with thiamine. In the presence of thiazole alone, there was no evidence of synthesis of pyrimidine.

Pellicularia koleroga Cooke

Several morphologically identical isolates were grown on synthetic media by Mathew, but none of them was capable of growing in this substratum unless thiamine or its two moieties were supplied in combination. Some of them responded to pyrimidine and thiazole separately as well as to riboflavin and biotin. Differences were noticed between isolates in their response to these growth substances which may be due to changes in the isolates in culture resulting in loss of their original capacities for synthesis.

Similarly, different isolates had varying capacities to grow in media containing riboflavin, biotin and pantothenic acid.

On repeated subculturing in artificial media, all isolates became more and more dependent on thiamine, while response to the other growth substances was reduced. However, a progressive reduction in the rate and amount of growth was noticed even in the presence of thiamine.

The optimum amount of thiamine for the growth of the fungus determined at the period when the fungus reached maximum growth was found to be near 1·0 μg./20 ml. of medium. Comparatively large amounts of biotin and riboflavin were found necessary to promote some growth of the fungus, especially after long maintenance in artificial media.

Additions of other growth substances to media containing optimum amounts of thiamine had no effect, but casein hydrolysate increased growth considerably and the growth of the fungus was not inhibited by excessive doses of thiamine and biotin, whereas riboflavin in similar doses depressed growth.

Piricularia spp. *and Sclerotium oryzae*

Piricularia oryzae Br. & Cav., *P. setariae* Nisikado, *P. zingiberi* Nisikado and *Sclerotium oryzae* Cattaneo, investigated by S. Suryanarayanan, indicate that, whereas all four fungi were heterotrophic for thiamine, *Piricularia oryzae* was unable to use either thiazole or pyrimidine in its early stages of growth but an equimolar mixture of both was as good as thiamine. Nevertheless, it was able to grow with pyrimidine as the incubation period advanced. This, however, was not the case with *P. setariae* and *P. zingiberi* which needed both fractions of the thiamine molecule. *Sclerotium oryzae*, on the other hand, did not utilize thiazole but grew with pyrimidine, completing the synthesis of thiamine. Thus, *Piricularia oryzae* in its early stages of growth, *P. setariae* and *P. zingiberi* have only the power to combine the thiamine moieties like *Phycomyces blakesleeanus*. However, *Piricularia oryzae* in its later stages of growth resembles *Sclerotium oryzae* in completing the synthesis of thiamine from pyrimidine alone.

All the species of *Piricularia* and isolates from *Eleusine coracana*, *Triticum vulgare* and *Brachiaria* are all biotin-deficient, the deficiency being complementary. *Sclerotium oryzae*, on the other hand, is autotrophic to biotin. This work does not confirm the results of Otani (1952), inasmuch as he reported that *Piricularia oryzae* is heterotrophic for biotin rather than for thiamine. However, the results obtained by Suryanarayanan confirm the earlier work of Leaver, Leal & Brewer (1947) on *P. oryzae* and indicate that the *Piricularia* spp. studied are autotrophic for biotin.

HEAVY METALS

The ability of some fungi to utilize minute traces of some heavy metals has been taken advantage of in bio-assay of these metals in soils, plant materials, chemical compounds, culture media, etc. (Mulder, 1939; Steinberg, 1945; Nicholas & Fielding, 1947, 1949, 1950, 1951; Nicholas, 1950; Hewitt & Hallas, 1951; Donald, Passey & Swaby, 1952*a*, *b*). The sensitivity of these organisms has, however, varied; but in most cases where they have been used, the accuracy of the quantitative estimation of the elements has been greater than the results obtained by chemical analysis (Nicholas & Fielding, 1951; Hewitt & Hallas, 1951). In this laboratory, the purity of the culture medium to be used in trace element essentiality studies has been tested biologically by Miss L. Saraswathi Devi using the standard 'M' strain of *Aspergillus niger*. Simultaneously, a strain of the same fungus, isolated in this laboratory was compared with the standard 'M' strain, as regards its sensitivity. The Indian isolate has been designated as strain 'M.U.B.L. 1'. This strain appeared to be less sensitive to traces of manganese, and more sensitive to copper and perhaps molybdenum also than the 'M' strain. Comparing the two strains of *A. niger* used in this study the responses to iron and zinc deficiencies of both the strains were nearly the same, although the 'M.U.B.L.' strain showed slightly greater sensitivity than the 'M' strain. The most obvious contrast between the two strains was shown in the Mn-, Cu- and Mo-deficient cultures. The nature and amount of growth and sporulation shown by the standard strain in the '– Mn' culture, though not normal, showed the presence of a certain amount of this element in the purified medium. In spite of this being so, the M.U.B.L. strain showed very marked deficiency symptoms; sporulation was completely suppressed and the pure white mat was much wrinkled, giving the 'cauliflower' appearance (Donald *et al.* 1952*b*). Hence, it seems evident that this strain requires a greater supply of manganese for normal metabolism than the standard strain. In other words, the new strain is less sensitive to traces of manganese than the 'M' strain. (The greater dry weight shown by the 'M.U.B.L.' strain in the Mn-deficient culture, in spite of marked, visual deficiency symptoms, is a point of interest.)

The reverse was the case in the '– Cu' and '– Mo' series, the standard strain showing definite deficiency symptoms in both the deficient cultures. The mat was entirely white and cottony, and sporulation completely suppressed except for a ring of black spores at the edge of the mat in the '– Cu' series where the fungus was in contact with the culture flask. At the same time, the laboratory strain showed better growth and sporulation. This was very marked in the '– Mo' series where the dry weight was even greater than that of the control, which is again an interesting feature. The probabilities are that, either the new strain does not depend on a supply of molybdenum for good growth and sporulation, or it is able to utilize to ts best advantage the minute traces of this element which have been left over in the medium due to insufficient removal (as shown by the somewhat thick pellicle of the standard strain in the '– Mo' culture), possibly ndicating a greater sensitivity of this strain to traces of molybdenum.

In the '– Cu' series, both the strains showed deficiencies, indicated by dry weight as well as spore intensity. Sporulation was almost suppressed n the 'M' strain—a definite deficiency sign. Still, the laboratory strain howed a scant cover of yellowish brown spores, and a greater dry weight oo, than the 'M' strain. It may, most probably, be that it is able to utilize copper in even smaller amounts than the standard strain and it is, herefore, very likely that the new strain is more sensitive to small traces of copper.

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THE DEVELOPMENT OF THE ASCOCARP OF *CYTTARIA GUNNII* BERK.

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(With Plates 16 and 17)

The fruiting habit and the development of the ascocarp of *Cyttaria gunnii* Berk. is described. The development of the ascocarp is angiocarpic. No ascogonium or archicarp was found, but ascogenous hyphae develop from clumps of thick, short-celled, hyphal filaments formed beneath the peridium-like rind in the solid primordial ascocarp. The mature ascocarp is a hollow pear-shaped stroma with many separate apothecia covering the upper two-thirds of the structure. These apothecia arise from the separate clumps of ascogenous tissue, and are connected during their development, by funiculae of hyphae which form a common columella-like structure in the basal portion of the stroma. In the early stages these hyphal strands are surrounded by a matrix of mucilaginous hyphae which gelatinizes as the ascocarp matures. The asci are inoperculate. The systematic position of *Cyttaria* is discussed.

INTRODUCTION

he Cyttariaceae, a family of Discomycetes confined to the southern emisphere, comprises parasites on southern beech, *Nothofagus* species, rowing in eastern Australia, Tasmania, New Zealand and South America. here may be some near relatives of this family occurring in Malaya on ood and bamboo stems (Boedjin, 1934).

In spite of early work on this family by Berkeley (1845), Fischer (1888), alm (1932) and others, there is little information concerning the develop- ent of the ascocarp, two aspects of which need clarification. The first oncerns the origin of the apothecia, and the second is whether the asci re operculate or inoperculate, since there is some doubt about the syste- atic position of this family.

There are two species of *Cyttaria* recorded for Australia: *C. gunnii* Berk. n *Nothofagus cunninghamii*, Hook. in Victoria and Tasmania; and *Cyttaria eptentrionalis* Herb. on *Nothofagus moorei* F. v. M. in New South Wales, and outhern Queensland, first described by Herbert (1932).

MATERIALS AND METHODS

he material for this study was *Cyttaria gunnii* Berk. growing on *Nothofagus unninghamii*, and was collected in early December (early summer) in 1945, t Tewkesbury in northern Tasmania. Histoid galls carrying all stages of evelopment of the ascocarp including the ripe stage were collected. Some f the material was dissected and drawings made within a day or so after ollecting. All sizes of ascocarps were fixed in chromacetic fixative and mbedded in wax for microtome sectioning. Some material was first fixed n formal-acetic-alcohol and then kept in 10% glycerine with phenol added as a preservative for additional dissections at a later stage. Microtome sections were stained either with carbol fuchsin or gentian violet and orange G. Hand sections from fresh and preserved material were stained in lacto-phenol cotton blue.

FRUITING HABIT

In Tasmania, the ascocarps (or ascophores) develop on histoid galls during early- and midsummer, that is, from the end of November till February, though ascocarps have been found as late as April. The woody histoid galls are rounded in form and vary in size from a few millimetres in young galls to 30 cm. or more in old galls on thick branches or on the trunk of the tree. These galls may prevent further growth of a branch, but are frequently lateral outgrowths of rounded form on stems and branches.

Each gall bears a variable number of ascocarps at different stages of development; the number usually depending on the size of the gall, e.g. one gall 10 mm. in diameter had fifteen ascocarps attached to it; another 16 mm. in diameter had twenty-five ascocarps. These ascocarps varied in diameter from 2 to 40 mm. At maturity each ascocarp is about 40 mm. in diameter, and they become fairly closely packed as shown in Pl. 16.

It appears that each gall produces an annual crop of ascocarps. The fruiting season coincides with the period of new growth of the host. In old beech forests enormous numbers of ascocarps are produced and after ascospore discharge, sometimes before, they fall to the ground forming dense carpets. The writer recalls walking on a track through a beech forest near Geeveston, Tasmania, covered to a depth of 4–6 in. with ascocarps of *Cyttaria*.

DEVELOPMENT OF THE ASCOCARP

The ascocarp begins its development on the surface of a gall as a pale cream-coloured stromatic button or papilla, about 1 mm. in diameter. It arises from a stromatic mass of hyphae located beneath the periderm that covers the gall. The mycelium from which it arises is perennial and is confined to the parenchymatous tissue of the gall as was shown for *C. septentrionalis* by Wilson (1937).

In section this stromatic button consists of an outer peridium-like rind containing a dense mass of hyphae with central wavy strands of deeply staining hyphae. The stromatic button enlarges distally to assume the pear shape which persists throughout its development. When about 2·0 × 1·5 mm., internal differentiation of the tissue begins. Numerous, separate, deeply staining clumps of thick short-celled hyphae are formed just beneath the peridium or rind, on the upper one-third of the developing ascocarp. These are the apothecial initials and are connected by funiculae of deeply staining thin-walled hyphae to a central columella-like structure also staining deeply. This columella seems to be formed from the wavy strands of hyphae first seen. The hyphal cells of both columella and funiculae are the same in appearance, are thin-walled and richly protoplasmic, and do not resemble ascogenous hyphae. The remainder of the interior of the ascocarp is filled with thick-walled mucilaginous hyphae which will be seen later to gelatinize and produce the hollow cavity in the interior of the mature ascocarp.

By the time the ascocarp is about 4 × 3 mm. each apothecial initial has differentiated into ascogenous hyphae at the base of a palisade layer of slender paraphyses which lines the small cavity in each apothecial initial. The funiculae appear to have enlarged and are hollow but still maintain their connexions with the developing apothecia and columella. The mucilaginous hyphae show cleavages at the beginning of gelatinization (Pl. 17, fig. 1).

In any one ascocarp, all apothecia show the same amount of differentiation, though each arises as a separate unit within the fruiting body.

Enlargement of the ascocarp takes place mainly in the distal region and, as a result of this enlargement, the apothecia come to occupy the upper two-thirds of the mature ascocarp.

When the ascocarp is about one-third its ultimate size, primary asci appear amongst the paraphyses in the palisade layer (Pl. 17, fig. 4). Gelatinization of the mucilaginous hyphae is most noticeable in the lower half of the ascocarp (Pl. 17, fig. 2). The hyphae of the funiculae and columella still stain deeply, though the central cavities of the funiculae have enlarged (Pl. 17, figs. 5, 6).

With the further enlargement to the mature size, gelatinization of the mucilaginous hyphae is completed, leaving a cavity behind the fused walls of the separate apothecia. The funiculae separate from the apothecia or gelatinize. The columella now contracts to a knob-like mass of tissue attached to the inner wall. Its position varies with different ascocarps. In some it is located at the junction of the lower sterile one-third part of the ascocarp and of the fertile part. In others it is high up inside the fertile part.

The peridium or rind covering the whole of the ascocarp is still intact, though there is some evidence that the tissue is breaking down over the cavities of the apothecia.

During this threefold enlargement ascosporogenesis takes place, and the palisade layer lining the apothecium consists largely of asci with slender paraphyses scattered amongst them (Pl. 17, fig. 3).

By the time the peridium or rind is beginning to break down over the apothecia, many of the asci contain eight nearly mature ascospores. These are globose and serially arranged in the ascus. No operculum is distinguishable at this stage of development but a refractive ring can be seen at the apex of each ascus (Pl. 17, fig. 6).

With the maturation of the ascocarp the peridium covering the cavities of the apothecia breaks down through gelatinization. Gradually each apothecium is exposed, those nearest the apex usually being first, until all cavities of the apothecia are open to the surface (Pl. 16).

There are many apothecia in each ascocarp though their number may vary. On two ascocarps there were 124 and 147 respectively.

The colour of the developing ascocarp is pale orange from the time it is about one-third of its mature size. When the apothecia are exposed the hymenium appears deep orange in colour. In sections of fresh material mounted in water it was found that hyphae in the subhymenial layer were filled with carotin-like pigment and this was responsible for the colour of the ascocarps.

Ascosporogenesis

Throughout the development of the ascocarp no structure resembling either an ascogonium or archicarp was found. However, soon after the differentiation of the hyphal clumps beneath the peridium, typical dicaryotic ascogenous hyphae with crozier formation could be distinguished.

Though no detailed cytological examination was made, ascosporogenesis appeared to follow the normal sequence of events known for the Pezizales.

Ascospore discharge was not observed, but the pore with its refractive disk could be seen at the apex of asci in mature apothecia both before and after rupture of the peridium.

Discussion

Earlier views on the systematic position of *Cyttaria* are reviewed by Palm (1932), who in discussing the new genus *Cyttariella* as a conidial stage of a species of *Cyttaria*, points out striking resemblances to members of the Sphaeriales. The perfect stage of *Cyttaria* leaves no doubt that it belongs to the Discomycetes. Recently the doubt has been whether the family Cyttariaceae belongs to the *Operculatae* or the *Inoperculatae*. Martin (1941) places the family in the operculate Discomycetes in the order Pezizales along with the families Helvellaceae and Pezizaceae. Bessey (1950) places Cyttariaceae amongst the inoperculate families of the Pezizales in his key based on Nannfeldt (1932). The asci of *Cyttaria gunnii* are shown here to be inoperculate, so that the proper systematic position of the Cyttariaceae is amongst the inoperculate Discomycetes.

It was hoped that a knowledge of the ontogeny of the Cyttariaceae might give more accurate data on its natural relationship with other members of the Discomycetes. This work indicates that its ontogeny is unique amongst the Discomycetes so far reported. In its angiocarpic development *Cyttaria* resembles some of the Discomycetes, namely the Phacidiales and the Tuberales. In this respect it also resembles the inoperculate family Patellariaceae placed in the Helotiales by Martin (1941), where the hymenium is formed angiocarpously, e.g. in the lichens *Usnea* and *Physcia*. Amongst the Pezizales, McCubbin (1910) reports that in the Helvellaceae the ascocarp in an early stage of its development is 'surrounded by an envelope' which is only transitory and 'breaks up irregularly'. From the text illustrations this envelope does not resemble the peridium-like rind of *Cyttaria*.

The development of hyphal funiculae connecting the developing apothecia with a centrally placed columella-like structure has not been reported for any of the other members of the Discomycetes, and appears to be unique for *Cyttaria*. In appearance this structure resembles the funiculus developed by the Nidulariaceae and the receptacle developed by the Phallales in the Gasteromycetes. However, it differs from them in that with *Cyttaria* the funiculae and columella function only during the development of the fruiting body. Palm (1932) in describing *C. intermedia* states that 'the stroma is solid during all stages of development observed, and shows a central, very characteristic dark-coloured vein or strand of brown-coloured hyphae from which thinner veins are branching off'.

It is probable that all species of *Cyttaria* have this columella with its hyphal funiculae. It is suggested that they function as conducting channels for nutrients, since they disappear after the apothecia have matured.

It seems then that the ontogeny of *Cyttaria* emphasizes the unique position of this family amongst the Discomycetes. It is interesting to speculate on the possibility of the antiquity of the Cyttariaceae in relation to their geographically isolated host, *Nothofagus*. This is to suggest that the Cyttariaceae may be the only surviving members of a much wider group of Discomycetes with a similar ontogeny. They have survived because their host has survived.

The fruiting season of *Cyttaria gunnii* coincides with the period of new growth of the host plant. Wilson (1937) observed in *C. septentrionalis* that the hyphae of the fungus are never present in primary wood of its host *Nothofagus moorei*; and suggests that infection of new growth occurs in early summer and that the mycelium remains dormant in or near the cambium until the beginning of the second year's growth. From a routine examination of the galls of *N. cunninghamii* caused by *Cyttaria gunnii* the writer suggests the same would hold for *C. gunnii.* Spore discharge, infection phenomena, and subsequent host-pathogen relations of the Cyttariaceae on southern beech need investigation.

Neither ascogonia nor archicarps are found in the developing ascocarps of *C. gunnii.* It is therefore suggested that the ascogenous hyphae which develop are parthenocarpic in origin. Further evidence on this aspect may be sought in cytological studies, which were not attempted in this work. Spermagonia are developed on the young ascocarps of some of the South American species of *Cyttaria* (Palm, 1932). However, the presence of spermagonia and spermatia does not necessarily mean that a normal sexual process takes place.

The writer gratefully acknowledges permission by Michael Sharland of the Flora and Fauna Protection Board of Tasmania, to use his excellent photograph of *Cyttaria Gunnii* as Pl. 1. Also the writer acknowledges his indebtedness to the Department of Illustration of Sydney University for the photomicrographs.

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EXPLANATION OF PLATES 16 AND 17

Plate 16

Fruiting habit of *Cyttaria gunnii* Berk., showing the cluster of ascocarps at different stages of maturity on a gall of *Nothofagus cunninghamii* Hook. Natural size. Photograph by Michael Sharland.

Plate 17

Fig. 1. *Cyttaria gunnii.* Lateral section of ascocarp at 4 × 3 mm., showing the rind-like peridium covering the separate apothecia that have arisen beneath it. The central columella with strands connecting the apothecia; and the mucilaginous hyphae filling the ascocarp. × 20, free-hand section.

Fig. 2. Lateral section of ascocarp about one-third of its ultimate size, with apothecia occupying about two-thirds of the ascocarp. Note gelatinization of the mucilaginous hyphae in the lower half of the ascocarp and the deeply staining columella and funiculae. × 20.

Fig. 3. Section of hymenium of a mature apothecium showing asci with refractive ring at apices and the slender paraphyses amongst the asci. × 420, stained with iodine solution.

Fig. 4. Section of hymenium of fig. 5 showing primary asci amongst the palisade layer of paraphyses. × 120.

Fig. 5. Transverse section of ascocarp, the same stage as in fig. 2, showing the peridium apothecia and hollow funiculae in the matrix of mucilaginous hyphae. × 20.

Fig. 6. Enlargement of portion of hollow funiculus, showing densely staining hyphae lining it and surrounded by thick-walled mucilaginous hyphae. × 420.

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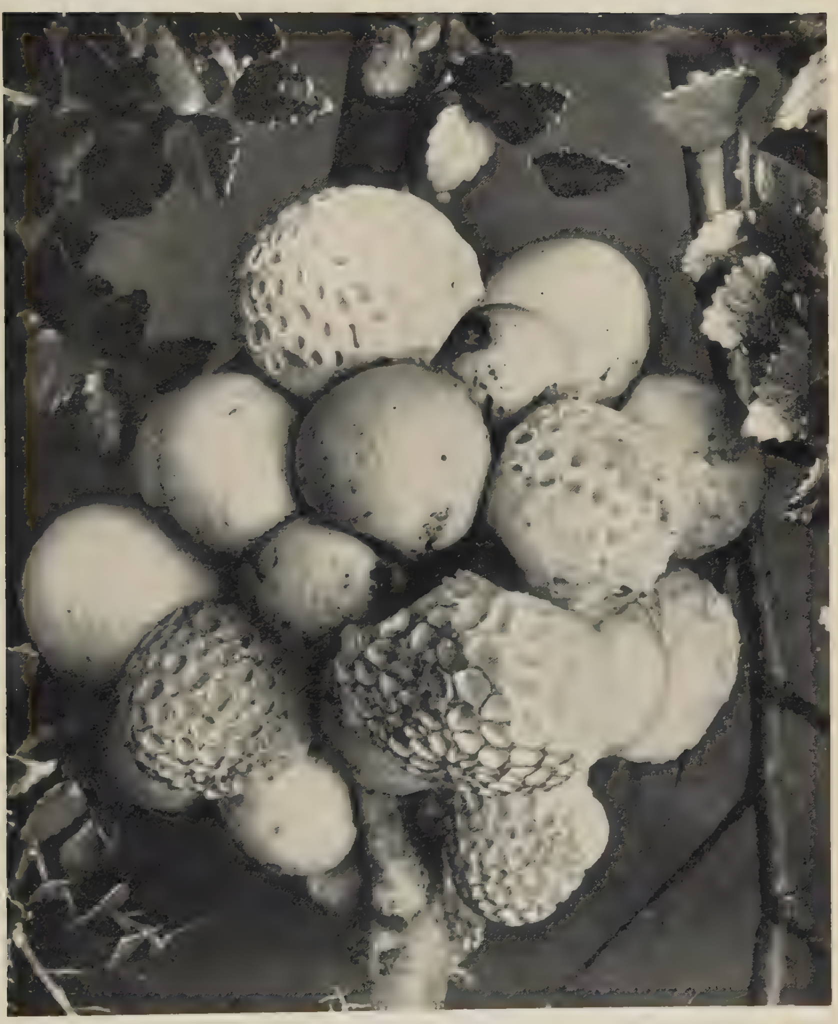

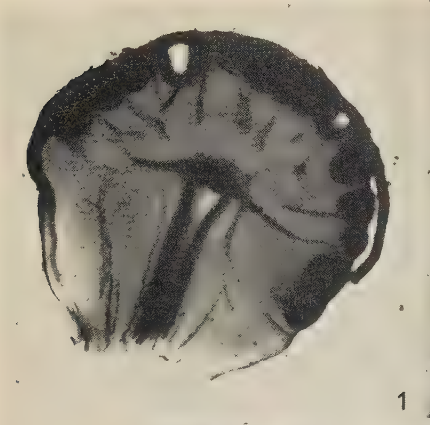
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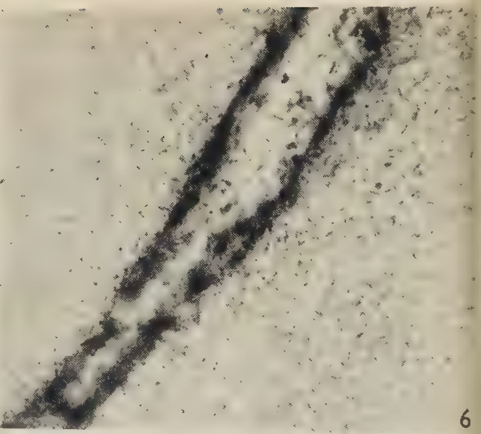
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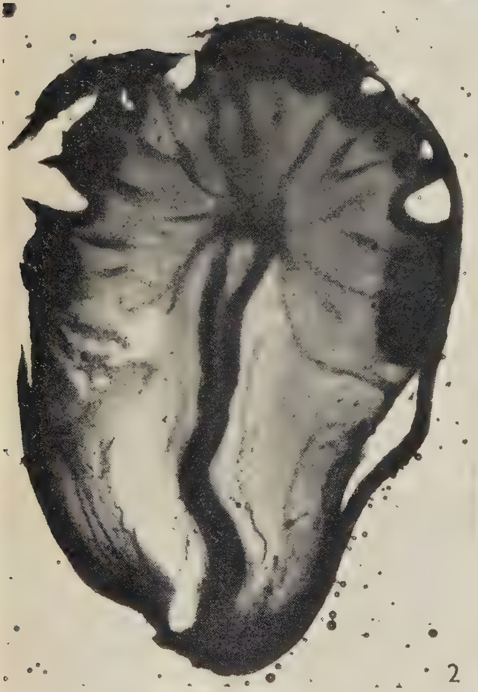
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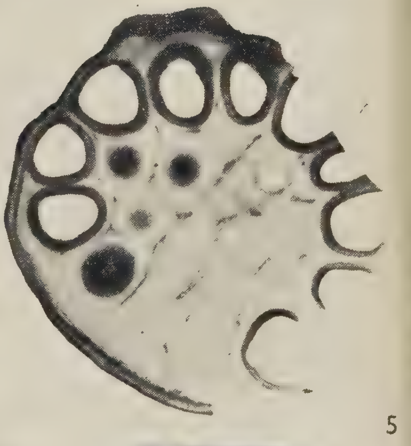
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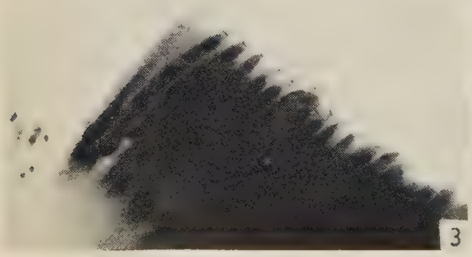
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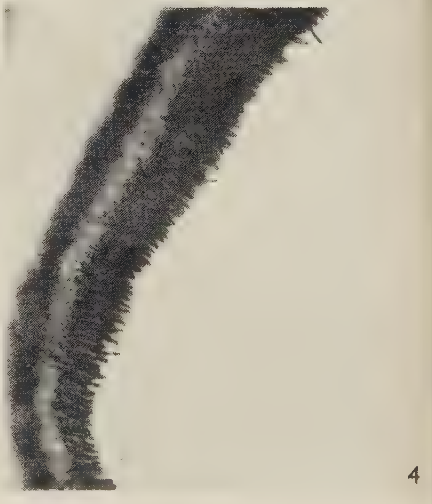
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DACTYLARIA PYRIFORMIS SP.NOV.

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(With 2 Text-figures)

This new predacious hyphomycete captures free-living nematode worms by means of sticky networks and produces heads of pyriform conidia.

Dactylaria pyriformis sp.nov.

Mycelium hyalinum, septatum, ramosum, 2·5–4·5μ crassum, reticula tribus dimenionibus et cellis 5–9μ latis evolvens, quibus vermiculi nematodei haerent. Hyphae e eticulis crescentes et viscera animalium edentes. Hyphae fertiles, hyalinae, septatae, erectae, 150–500μ altae, 4–10μ latae in base, contrahentes ad 2·5–4μ, in apice ad 16 (plerumque 4 aut 5) conidia in forma capitis ferentes. Conidia hyalina, longe-pyriormia, obtusa, plerumque bi- aut tri-septata, raro uni-septata, 26–41μ longa et 9–14μ rassa in loco latissimo. Chlamydosporae intercalares, singulae, paullum silaceae, globosae, ellipsoidales, 20–35μ longae et 10–25μ crassae.

Vermiculos nematodeos capiens et consumens in acervo stercoris in Nocton Fen, Lincolnshire, Anglia. May 1953.

This fungus was first observed capturing nematode worms on 13 May 1953 on maize-meal agar plates which had been inoculated with small pieces of manure taken from a dung heap at Nocton Fen, Lincolnshire.

The mycelium consisted of colourless, branched, septate hyphae, 2·5–4·5μ wide, growing out from the inoculum and producing three-dimensional networks at intervals. These networks had cells 5–9μ in diameter (Fig. 1 *a*). Nematode worms appeared to stick to the networks and were usually unable to detach themselves in spite of their violent truggles. Eventually the fungus produced septate, endozoic hyphae, which assimilated the soft parts of the eelworms (Fig. 1 *b*, *c*). Capture of a relatively large nematode worm seemed to stimulate the fungus to produce abundant networks around the captive, and smaller or juvenile worms were captured by this extensive hyphal meshwork. After a period of predacious activity the fungus produced erect, septate, colourless conidiophores 150–500μ high, 4–10μ in diameter at the base tapering upwards o 2·5–4μ at the tip. At the tip of each conidiophore there developed a head of conidia containing up to sixteen (usually four or five)spores (Fig. 1 *d*). Growing on the agar plate the conidiophores with the terminal heads of conidia resembled those of an *Arthrobotrys* when viewed with the low-power objective of the microscope. The conidiophores were usually simple, but ometimes they were simply branched and carried more than one conidial head due to continued growth of the conidiophore. The conidia were hyaline, elongate-pyriform in shape with broadly rounded distal ends; hey were 26–41μ long and 9–14μ in greatest width. The conidia were usually bi- or triseptate, but bicellular spores were very occasionally seen

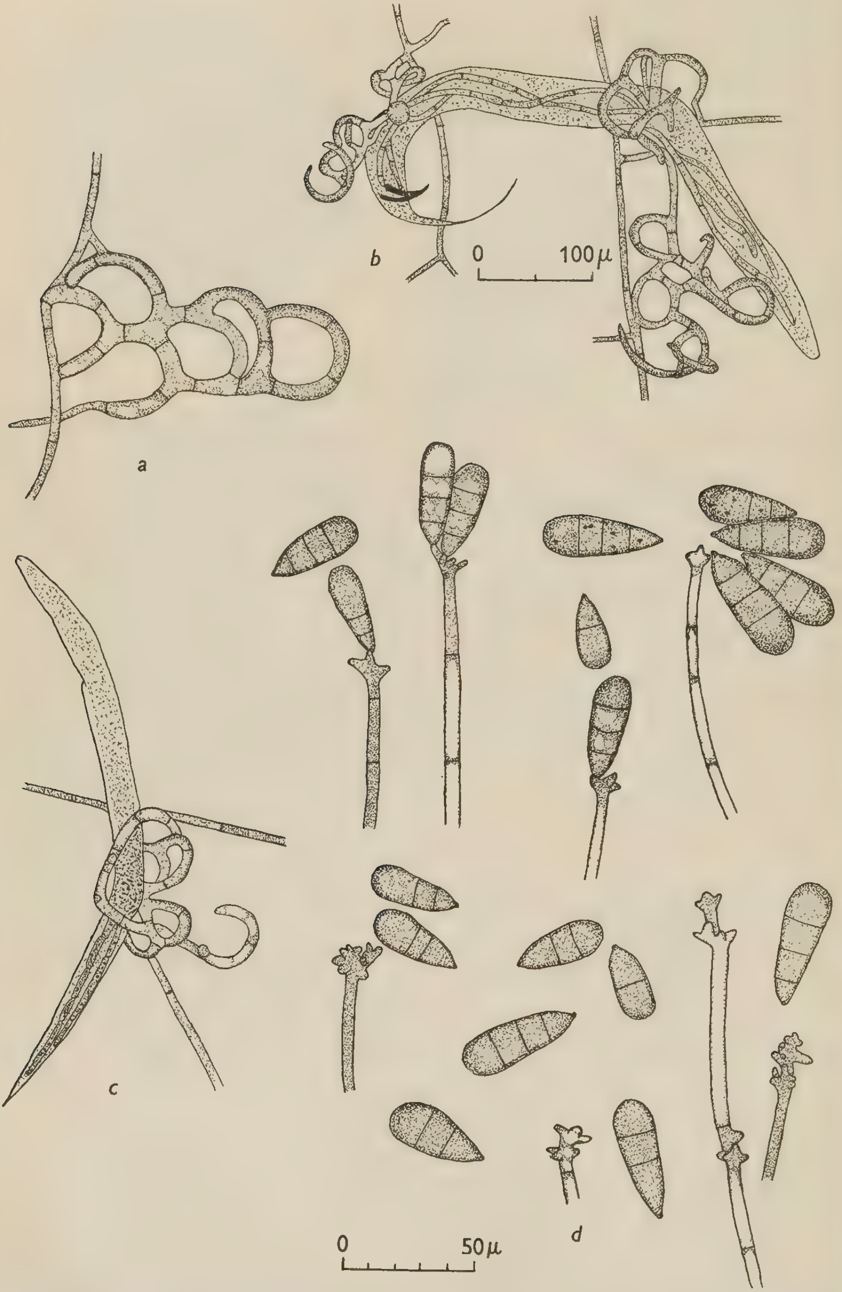

Fig. 1. *Dactylaria pyriformis* sp.nov. *a*, a three-dimensional hyphal network; *b*, *c*, nematode worms captured by the fungus; *d*, tips of conidiophores and conidia.

(Figs. 1 *d*, 2 *a*). The conidia germinated freely on the surface of the agar, germ tubes developing from any cell of the spore (Fig. 2 *b*). After a period of predacious activity and sporulation the fungus produced intercalary chlamydospores. These were thick-walled, globose or ellipsoidal in shape, measuring 20–35 μ × 10–25 μ, yellowish with dense globuliferous contents (Fig. 2 *c*).

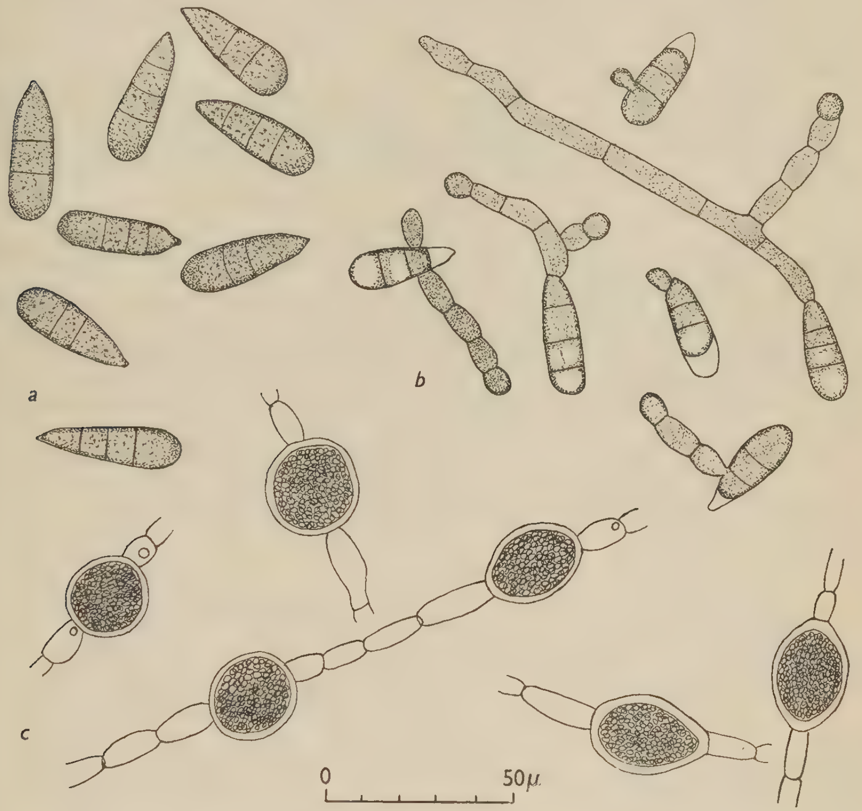

Fig. 2. *Dactylaria pyriformis* sp.nov. *a*, conidia; *b*, germinating conidia; *c*, chlamydospores.

The fungus was isolated and grew very well on maize-meal agar. In pure culture it failed to produce the nematode-capturing traps but sporulated very freely. In pure culture the conidia measured 20–40 × 8·5–13·5 μ, uniseptate forms being less rare. After being kept on maize-meal agar slopes for 9 months some of the fungus was placed on to sterile maize-meal agar plates. A week later small portions of an eelworm culture growing on rabbit dung agar were added to the plates, the fungus produced networks very readily and captured worms.

Five species of *Dactylaria* have been described which capture nematode worms by means of adhesive, three-dimensional networks. *D. thaumasia* Drechsler (1937) and *D. eudermata* Drechsler (1950) have top-shaped conidia, while those of *D. polycephala* Drechsler (1937), *D. psychrophila* Drechsler (1944) and *D. scaphoides* Peach (1952) are more or less fusiform in shape. The conidia of my fungus are quite different in shape from those of the species of *Dactylaria* which form networks hitherto described, and I have therefore described it as a new species, *D. pyriformis*, the specific epithet referring to the shape of the spores.

Type material will be deposited in the Herbarium of the Royal Botanic Gardens, Kew, and in the Herbarium of the Commonwealth Mycological Institute, Kew.

My thanks are due to Mr M. Brook who very kindly sent me the manure sample from Nocton Fen, to Mr J. A. Easterling for help with the Latin diagnosis, and to Dr C. L. Duddington for his invaluable help and advice.

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REVIEWS

An Introduction to Industrial Mycology. By George Smith. 4th edition. (London: Edward Arnold. 1954.) xiv + 378 pp., 161 text-figures. Price 30*s.*

ince its first appearance in 1938, there have been three editions of this book and the hird edition, published in 1946, was reprinted in 1948. In this fourth edition the text has been completely revised and much of it rewritten; numerous illustrations have been added and some of the old ones replaced. The book is designed primarily to introduce students and others with little or no botanical knowledge to the study of a large number of the micro-fungi which are of importance in industry. Rather more than half the book s devoted to classification and descriptions of fungi, and the rest to laboratory equipment and technique, the physiology of mould fungi, the maintenance of culture collections, control of mould growth, industrial uses of fungi, and mycological literature; there is a new Appendix which deals with microscopy. The list of special terms on pages 10–12 might usefully be expanded to include a few others such as 'acervulus' and 'apothecium'. The book is illustrated by 160 most excellent photographs and one line drawing (after Mangin). The method of attachment of conidia is often only vaguely indicated in the photographs, e.g. in *Cephalosporium* (p. 105), *Oidiodendron* (p. 124), *Trichoderma* (p. 135) and *Trichothecium* (p. 136); these would be better illustrated by line drawings. In most ections references to literature are quite up to date; no mention is made, however, of he excellent work done by Snyder and Hansen on *Fusarium*, and by Steyaert on *Pestalotia* and *Pestalotiopsis*. In most mycological text-books very little space is devoted to the Fungi Imperfecti, although economically they are one of the most important groups of ungi; it is refreshing to read one in which they are treated in the right perspective. Mr Smith's book has probably already given more people a lively introduction to the moulds' than any other, and it will undoubtedly continue to be read with pleasure for many years to come.

M. B. ELLIS

The Polyporaceae of the United States, Alaska and Canada. By L. O. Overholts. Prepared for publication by Josiah L. Lowe. (Ann Arbor: University of Michigan Press. London: Geoffrey Cumberlege. 1953.) xiv + 466 pp., 132 uncoloured plates. Price 60*s.*

At the present time the classification of the Polyporaceae, as indeed that of the Aphyllophorales in general, is more or less in the melting-pot. During the past fifty years here have been enormous advances in our knowledge of the microscopic anatomy of hese fungi, and modern workers tend to attribute more and more importance to microcopic characters and less to the gross morphological features used by the early mycoogists for the division of these fungi into families and genera. In the Polyporaceae as at present understood, (that is omitting *Boletus* and *Merulius*) Fries in his *Systema Mycoogicum*, 1 (1821) had but two genera, *Daedalea* and *Polyporus*. Out of *Polyporus* there have been segregated from time to time numerous genera. Fries himself later established *Polystictus* and *Trametes*, and accepted various other genera, but the large number of ater additions are due mainly to the work of Karsten, Quélet and Patouillard in Europe, and of W. A. Murrill in America. Murrill established a record with fifty-eight genera of Polyporaceae in the North American Flora, and at the same time complicated matters by using the American Code of Nomenclature. More conservative work in America was hat of Miss Ames and of Overholts, which is put together in the book under review.

We learn from the Preface that Dr Overholts wrote the first draft of this work in 1933, and that though he continually corrected the manuscript to incorporate new information he made no overall revision before his death in 1946. The classification therefore follows he same lines as that laid down by the author in his early papers on the Polyporaceae of Ohio and of the Middle-Western United States (1914 and 1915). He admits only ten genera, including *Merulius* and *Poria*, which are not dealt with in the present book; *Polystictus* is not recognized, nor *Ganoderma* Karst.; in fact, no genus established since 1867, when Kickx gave generic rank to Fries's section *Fomes*, has found favour with Overholts. The result is a very large and unwieldy genus *Polyporus*, with 162 American species. The classification used by Bourdot and Galzin is commended for those who would go further but do not like the excessive splitting of some authors, but the work of Donk and Pilát has not been considered, and there is no mention of the recent anatomical investigations of Corner and Cunningham. It is true that the value of this recent work from the taxonomic standpoint has yet to be tested, but to return to the early Friesian conceptions is hardly helpful at the present day. A student wishing to use the key to *Polyporus* given by Overholts is likely to find it difficult if not unworkable. On the other hand, he may be helped by the numerous excellent photographs showing the general appearance of the species. The 124 plates from photographs include 675 figures showing habit. In addition, there are eight plates with line drawings of hymenial features—spores, basidia, and setae or cystidia when present. A glossary and index complete the volume.

For the American student, who has had no complete account of American Polyporaceae since Murrill's work in the North American Flora, the book will undoubtedly be very welcome. For others the value of the book lies chiefly in the indications of Overholts's views as to certain species, and in the figures. E. M. WAKEFIELD

Les Genres Sordaria et Pleurage. Leurs affinités systématiques. By CLAUDE MOREAU. (Paris: Paul Lechevalier. 1953.) 330 pp., 79 figures. Price Frs. 3500. (French text.)

In the first chapter the history of the classification of the lagynocarpous Ascomycetes is reviewed, the author preferring this term to 'Pyrenomycetes'. He is of the opinion that habitat, colour, spore septation, presence or absence of a stroma are of relatively little importance as a basis of classification, but from his reading of the literature and from his own research considers that the structure and origin of the perithecial primordium, the ascus apex and the method of spore expulsion are the significant taxonomic criteria.

A résumé showing the existing varied and confused conceptions of the limits of the family Sordariaceae and its genera forms the second chapter.

Most of the fungi studied were fimicolous, and 157 samples of the dung of herbivorous animals were examined from different parts of France and also from the Ivory Coast, the Cameroons and Palestine. In an exhaustive study of *Sordaria fimicola*, emphasis is placed on the mycelial structure, the perithecium and its development and origin, the asci with precise morphology of the ascal tip, ascospores and ascospore expulsion. Detailed also is the cytology of the species and its biology including cultural characters and tables describing growth-forms on a variety of more or less natural media. There are also similar tables giving cultural descriptions of *S. humana*, *S. macrospora* and *S. papyricola*, the other species regarded by the author as being true Sordarias. The effects of stimuli of various kinds were also investigated, i.e. that of light and heat, of *Penicillium chrysogenum*, of bacteria, of pH and of other *Sordaria* spp. Another section of the book is devoted to genetical observations of the four species above mentioned and the ecology and systematics. Under the last heading a large number of other species, frequently described under the genus, are analysed taxonomically and excluded. A special study is made of the fungus commonly known as *Sordaria bombardioides* Auersw. and it is concluded that, in spite of its coprophilous nature, its ascus with apex similar to that of the type species, the variable number of spores and the presence of an ascospore capable of swelling, this species constitutes a special genus. The name proposed for it is *Bombardioidea*, to indicate its affinities also with *Bombardia*. In conclusion, *Sordaria* is thought to fall into two sections, one including *S. fimicola*, *S. humana* and *S. macrospora* and the second only *S. papyricola*.

A comparable investigation is made of *Pleurage* (*Podospora*), a much less homogeneous genus. Within a single species a number of varieties and forms may be distinguished according to the number of spores per ascus, the size of the spores, the character of the primary and secondary appendages. *P. decipiens* has been found with forms having 8, 16, 2 and 64 spores per ascus; one form of *P. fimiseda* has unusually large spores and in . *setosa* the author found all intermediates between primary-appendaged spores and ose without.

Pleurage forms a transition genus: species having no appendages approach *Sordaria*, hereas in other characters the genus near *Bombardia*. Four sections are distinguished: *urvula*, *anserina*, *decipiens* and *fimiseda*.

The genera *Hypocopra*, *Delitschia*, *Sporormia*, *Pleophragmia* and *Sporormiella* were also nvestigated, but in less detail. The conclusion is reached that they do not belong to the amily Sordariaceae, that *Hypocopra* is one of the Xylariaceae and that the other four enera are related to *Leptosphaeria* and *Pleospora*. He therefore limits the traditional ordariaceae to the two genera *Sordaria* and *Pleurage*. The final chapter is an examination f genera regarded as related: *Gelasinospora*, *Neurospora*, *Coniochaeta*, *Bombardia* and *riangularia*, all of which belong to Nannfeldt's Ascohymeniales Sphaeriales and con-itute, together with *Sordaria* and *Pleurage*, the author's conception of the family Sor-ariaceae, i.e. *sensu* Moreau. The family *sensu auct.* is regarded only as an aggregation of onverging forms.

Moreau's inclusion of *Hypocopra* with the Xylariaceae is in agreement with the work f Munk whose latest paper 'The System of the Pyrenomycetes' in *Dansk Bot. Ark.*, 1953, e was unable to examine. But the Danish author includes *Sordaria*, *Pleurage*, *Bombardia* nd *Coniochaeta* with the Lasiosphaeriaceae of v. Hoehnel. The two authors differ also their treatment of *Delitschia*: Moreau placing it in Pleosporeaceae and Munk regarding as aberrant.

The book, as all in this series, is well produced, has good figures but, like most French terature, is very highly priced. This is particularly regrettable as the work is an able nd very important study which cannot be overlooked.

F. L. BALFOUR-BROWNE

ome South African Edible Fungi (35 pp., 8 coloured plates) and a companion volume—*Some South African Poisonous and Inedible Fungi* (31 pp., 8 coloured plates). By E. L. STEPHENS and M. M. KIDD (with an introduction by A. A. Pearson). [Longman's Field Handbooks.] (Cape Town: Longman, Green and Co.) Price 6*s*. each volume.

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P. D. ORTON

redineana. Tome 4. Edited by A. L. GUYOT. 602 pp., plates and numerous text-figures. [*Encylopédie Mycologique*. Tome 24.] (Paris: Paul Lechevalier, 28 December 1953.) Price Frs. 9000.

his uredinologist's miscellany of original papers, abstracts, and bibliographical data is n similar lines to the previous volumes (see *Trans. Brit. mycol. Soc.* **35**, 93, 1952). The onger papers include G. B. Cummins on the species of *Puccinia* parasitic on the Andro-ogoneae of the world (pp. 5–89, 11 plates), I. Jørstad on the Pucciniastreae and elampsoreae of Norway (pp. 91–123), and an account of rusts from the Ivory Coast by . Viennot-Bourgin (pp. 125–228). There is also a further supplement to Guyot's *es Urédinées*, **1** and **2** (pp. 505–522).

G. C. A.

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INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, Cambridge University Press, 200 Euston Road, London, N.W. 1, price 2*s*. 6*d*.). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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